Sudan University of Science and Technology

Collage of Medical Laboratory Science

Department of Haematology and Immunoheamatology

Evaluation of PT and APTT in Umbilical Cord Blood of Gestational Diabetic Mothers

تقييم زمن الثرومبين والثرومبوبلاستين المنشط الجزئ في دم الحبل السري لدى الامهات اللاتي لديهن سكر الحمل

A thesis is submitted for partial fulfillment for the degree of M.SC. In haematology

Submitted by:

Nusiba Mohamed Mohamed Ahmed Hamd.

B.Sc Medical Laboratory Sciences University of Khartoum

2015

Supervisor:

Dr. Hiba BadrEldin Khalil, Ph.D

Associate Professor, Haematology & Stem Cell Technology

2018

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيم

قال تعالى : (*فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَى إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْمًا*)

صدق الله العظيم

سوره طه الآيه(114)

DEDICATION

TO MY BELOVED AND PLEASED PARENTS WHOM EVERY THINGS FOR ME.

TO MY WONDERFUL SUPERVISOR;

DR. HIBA BADRELDIN KHALIL WHO WAS WITH ME WHEN NEED.

TO MY SPECIAL FRIENDS AND COLLEAGUES WHO WERE INTEGRAL PARTS OF SUPPORT GROUP.

I DEDICATE THIS WORK

Acknowledgments

All great thanks are firstly to Allah who guided me to the straight way in my life.

I would like to gratefully thank Dr. **Hiba Badreldin Khalil,** My supervisor, for her patience, guidance and encouragement throughout the study.

My thanks extended also to Dr. Khalid Saad Mohamed El Fadil for his helpful suggestions, intellectual advice, and genuine guidance during my work.

Moreover, the help and support provided by technical lab staff of Alsaaha Specialized Hospital is gratefully acknowledged.

Moreover, I would like to express my special thanks to the staff of Saad Aboulela University Hospital and Al-Qma Specialized Hospital for their help in sample collection .

Finally my thanks extent to my colleagues and friends who participated in this study.

List of Contents

List of Tables

List of Figures

Abbreviations

Abbreviation	Full text
AA	Arachidonic Acid
ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
ADP	Adenosine Diphosphate
Ang	Angiotensin
AT	Antithrombin
ATP	Adenosine triphosphate
BMI	Body Mass Index
CaCl2	Calcium Chloride
CD	Cluster Designation
DIC	Disseminated Intravascular Coagulation
ECs	Endothelial Cells
EDRF	Endothelial-derived relaxing factor
FBG	Fasting blood glucose
FDPs	Fibrin Degradation Products
GDM	Gestational Diabetes Mellitus
GP	Glycoproteins
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes
IADPSG	International Association of Diabetes and Pregnancy Study Group
IGT	Impaired Glucose Tolerance
INR	International Normalized Ratio

IR	Insulin Resistance
ISI	International Sensitivity Index
LGA	Large for Gestational Age
NO	Nitric Oxide
NDDG	National Diabetes Data Group
NIH	National Institutes of Health
NPH	Neutral protamine Hagedorn
OGTT	Oral Glucose Tolerance Test
PDGF	Platelet Derived Growth Factor
PED	Pregestational Diabetes
PK	Prekallikrein
PPP	Platelets Poor Plasma
PTT	Partial Thromboplastin Time
PT	Prothrombin Time
RAAS	Renin Angiotensin Aldosterone
RT	Reptilase Time
T2DM	Type 2 Diabetes Mellitus
T1DM	Type 1 Diabetes Mellitus
TFPI	Tissue Factor Pathway Inhibitor
TF	Tissue Factor
TNF	Tumor Necrosis Factor
T-PA	Tissue Plasminogen Activator
TT	Thrombin Time
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

Abstract

Introduction: GDM affects 2–10% of women during their pregnancy. It is important to recognize and monitor GDM closely due to the risk of adverse effects on the mother and the fetus. Newborns of women with a history of GDM or risky gestation are also at increased long-term risk of developing metabolic diseases and macrosomia. Maternal glucose easily crosses the placenta and as a consequence maternal hyperglycemia leads to intrauterine hyperglycemia, which induces fetal hyperinsulinemia and possible modification of growth and future metabolism of the fetus. The association between hyperglycemia and thrombosis is well known, and thrombosis in fetal umbilical cord vessels rate of 1/250 in risky gestations. It was monitored that there was venous thrombosis in 70 % of the Cases determined to have had cord thrombosis, the casual mechanisms and related effects of thrombosis are not well understood. PT and APTT have been reported to be increased during pregnancy, but how this effect has clinical impact on newborn cord blood PT and APTT is still unexplained. This study aimed to assess PT and APTT level in cord blood of newborn in mothers with gestational diabetes mellitus.

Materials and Methods: This is a cross sectional descriptive study carried out in Khartoum state in Saad Aboulela University Hospital, Alsaaha Specialized Hospital and Al-Qma Specialized Hospital, in the period from March to June 2018. Thirty samples collected from cord blood of newborn of GDM and thirty matched control group from cord blood of newborn of healthy pregnant were recruited in this study. Citrated Blood sample was collected from each newborn cord blood of normal pregnant women as control for estimation of PT,INR and APTTby using CL-2000 Biobase automated coagulometer, Data was analyzed by SPSS version 20, and expressed as means and tables.

Results: The mean of PT was significant increased in cord blood of infant of GDM (19.61±4.47) when compared to the control group (15.42±1.67) (P. value= 0.000).Also the mean of INR was significant increase in newborn cord blood of GDM (1.64±0.45) compared to the control (1.17±0.16) In addition; the mean of APTT is also significantly increased in newborn cord blood of GDM (53.53±3.86) compared to control group (50.83±4.40) P-value 0.014**.** An insignificant increase in PT of cord blood from GDM pregnant with FBG <100 (mg/dl) (19.21±5.45) to GDM pregnant women with FBG>100 (mg/dl) (19.79±4.11).Also the INR was insignificant increase in cord blood from GDM pregnant with FBG <100 (mg/dl) (1.67±0.40) to GDM pregnant women with FBG>100 (mg/dl) (1.56±0.56). Otherwise an insignificant increase in APTT of cord blood from GDM pregnant with FBG <100 (mg/dl) (54.33±4.42) to GDM pregnant women with FBG>100 (mg/dl) (53.19±3.65).No correlations between PT ,INR and APTT level and duration of diabetes , BMI, history of diabetes, hypertension of women (P. value > 0.05). Furthermore, no association between PT ,INR and APTT level and newborns weight (P> 0.05).

Conclusion: The study concluded that PT, INR and APTT could be used as indicator for the risk of thrombotic abnormality of newborn for future management.

ملخص الدراسة

مقدمة: مرض سكري الحمل يصيب بنسبة 2-10% من النساء الحوامل. من المهم معرفة ومتابعة المرض تجنبا لحدوث التاثيرات العكسية لكل من الام والطفل. حديثي الولادة اللائي امهاتهن لديهن مرض السكري او مخاطر في الحمل سابقا تزداد لديهن اكثر مخاطر الامراض الايضية.يعبر السكر من دم الام الى المشيمة بسهولة وكنتيجة لذلك زيادة السكر في دم الام يؤدي الى زيادة السكر داخل الرحم،مما يؤدي الى زيادة الانسولين في الجنين ويعدل من نظام النمو لدى الطفل مستقبلا. العلاقة بين ارتفاع السكر في الدم وتجلط الدم معروفة، حيث ان تجلط الدم في دم الحبل السري للجنين يعادل 1\250 من مخاطر الحمل، وتم رصد تجلط الدم الوريدي في 70% من الحالات التي تحدد تجلط الدم في الحبل السري، والسبب الاساسي وآثار تجلط الدم لمم تفهم جيدا. تم التقرير مسبقا ان زمن البروثرومبين و زمن الثرومبوبلاستين المنشط الجزئ على انهما يزدادان خلال فترة الحمل، لكن كيف يمكن ان تؤثر هذه الزيادة لديها سريريا في دم الحبل السري لحديثي الولادة لم تشرح حتى الان. هذه الدراسة تهدف لقياس زمن البروثرومبين و زمن الثرومبوبلاستين المنشط الجزئ في دم الحبل السري لحديثي الولادة اللائي امهاتهن لديهن سكري الحمل.

طرق البحث: هذه دراسة مقطع عرض لحالة مقارنه بحالة ضابطة في ولاية الخرطوم في مستشفى سعد ابو العلا الجامعي ومستشفى الساحه التخصصي ومستشفى القمة التخصصي في الفترة من مارس٢٠١٨ الى يونيو٢٠١٨. تهدف الدراسة لقياس زمن البروثرومبين و زمن الثرومبوبلاستين المنشط الجزئ في الحبل السري لاطفال مرضى سكري الحمل.تم أختيار ثلاثين عينة من الحبل السري لاطفال مرضى سكري الحمل كحاله دراسه و ثلاثين عينة من الأصحاء كمجموعه ضبط، تم أخذ دم الوريدي من كل مريضه و حاله ضابطه لقياس زمن البروثرومبين و زمن الثرومبوبلاستين المنشط الجزئ بواسطه استخدام جهاز (C-2000 Biobase) وتم

تحليل النتائج بواسطة برنامج الحزم الاحصائية للعلوم الاجتماعية اصداره 20وتم توضيح النتائج في شكل متوسطات و جداول .

النتائج: :تم حساب متوسط النتائج كالتالي : متوسط نتائج للمرضى أن قيمه زمن البروثرومبين ،النسبة المعيارية الدولية و زمن الثرومبوبلاستين المنشط الجزئ على التوالي (19.61±4.47) (1.64±0.45)(53.53±3.86). أما بالنسبة للمجموعة الضابطة فقد كانت النتائج لنفس المتغيرات كالتالي: (15.42±1.67) (1.17±0.16) (50.83±4.40) هذه النتائج عند مقارنتها بالمجموعه الضابطه نجد ان هناك فروقات ذات دلاله احصائيه وزياده معنويه واضحه بين متوسط زمن البروثرومبين ،النسبة المعيارية الدولية و زمن الثرومبوبلاستين المنشط الجزئ لدى المرضى و المجموعه الضابطه(القيمة المعنوية اصغر من ٠،٠٥)، وأيضا عند مقارنة هذه الدلائل مع نسبة الجلكوز الصائم 100> نجد زيادة معنوية لكل من قيمه زمن البروثرومبين ،النسبة المعيارية الدولية و زمن الثرومبوبلاستين المنشط الجزئ على التوالي(5.45_+19.21) (0.40_+1.67) (4.42_+54.33) أما بالنسبة للمجموعة الضابطة فقد كانت النتائج لنفس المتغيرات كالتالي:(4.11_+19.79) (0.56_+1.56) (3.65_+53.19) كان هناك فروقات ذات دلاله أحصائيه في زمن البروثرومبين ،النسبة المعيارية الدولية و زمن الثرومبوبلاستين المنشط الجزئ لدى المرضى حسب حده المرض (القيمة المعنوية اصغر من ٠،٠٥).

الخلاصة:اشارت المحصلة على ان فحص متوسط زمن البروثرومبين ،النسبة المعيارية الدولية و زمن الثرومبوبلاستين المنشط الجزئ يمكن ان يستخدم كمتنبئ مفيد للجلطات لدلا اطفال مرضى سكري الحمل.

Chapter One

1. Introduction

1.1Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of various degrees that is first detected during pregnancy. GDM is detected through the screening of pregnant women for clinical risk factors and, among at risk women, testing for abnormal glucose tolerance that is usually, but not invariably, mild and asymptomatic. GDM appears to result from the same broad spectrum of physiological and genetic abnormalities that characterize diabetes outside of pregnancy. GDM is a form of hyperglycemia. In general, hyperglycemia results from an insulin supply that is inadequate to meet tissue demands for normal blood glucose regulation (Thomas and Anny, 2005).

1.1.1 Classification

Uniform classification of diabetic in pregnancies is still needed for both epidemiological and clinical purposes. Both the World Health Organization (WHO) and National Diabetes Data Group (NDDG) of National Institutes of Health (NIH) have endorsed a classification based on the etiology. WHO classification differ only by recognizing impaired glucose tolerance (IGT) before pregnancy. This is simple but of no prognostic value.

Classification of maternal diabetes in pregnancy:

- Pregestational diabetes: preexisting type1 or type2 or secondary.
- Gestational diabetes: diagnosis is made post gestationally, normal glucose tolerance.

The classic classification system of diabetes in pregnancy was initially developed by Dr. Priscilla White in 1949 and referred currently as the White's classification. On the basis of age at onset, diabetes duration, metabolic, and vascular complications, Dr. White divided diabetes in pregnancy in classes from "A" (more favorable) to "F" (less favorable). The original White's classification underwent multiple modifications, until 1980. The first revision was done in 1965 by shifting vascular complications to "D" and adding class "R" which denotes the presence of proliferative retinopathy. In 1972 a further update was made in which, GDM was included in class "A" and class "D" was subdivided into five categories. The latest modification applied to the White's classification includes addition of GDM as a distinct separate class and deletion of class "E" and "G". The American College of Obstetricians and Gynecologists (ACOG) proposed another classification for GDM, adding a note for the presence or absence of metabolic complications, doubting the usefulness of the White's classification in clinical practice.

Currently, the term diabetes in pregnancy has been suggested to include all cases of hyperglycemia observed during pregnancy comprising GDM

and PED. The latter include pre-gestational type 2 diabetes mellitus (T2DM) and type 1 diabetes mellitus (T1DM), and GDM is defined as any degree of hyperglycemia that is recognized for the first time during pregnancy. This definition of GDM should be understood as to include cases of undiagnosed T2DM *"overt diabetes"* identified early in pregnancy and true GDM which develops later in pregnancy (Chamberlain *et al*., 2013).

1.1.2 Epidemiology

The prevalence of GDM has been progressively increasing and it reflects the background prevalence of obesity and T2DM in general population. Higher rates of GDM were found to raise in parallel with higher rates of T2DM. This may be related to the common risk factors including obesity, physical inactivity, ethnic background and urbanization. In Northern Californian pregnant women without pre-existing diabetes, reported that the prevalence of GDM was low among non Hispanic white women and African Americans, and high in Asians and Filipinas. Interestingly higher rates of GDM were demonstrated among those with lowest BMI (Asians and Filipinas) and lower rates were found in those with highest BMI (non-Hispanic white women and African Americans)(Hedderson *et al*., 2012).

1.1.3 Etiology

Pregnancy represents a complex metabolic and physiological condition that can be considered as a status of biological tolerance test which has the ability to detect insulin resistance earlier. Insulin resistance (IR) in pregnancy could be the result of maternal obesity with varying degree of adipocytokine production, or increased production of diabetogenic placental hormones. In addition to insulin resistance, pancreatic β-cell dysfunction might also play a role in the pathophysiology of GDM (Abdel Hameed and John, 2017).

1.1.4 Pathophysiology of GDM

Insulin resistance during pregnancy stems from a variety of factors, including alterations in growth hormone and cortisol secretion (insulin antagonists), human placental lactogen secretion (which is produced by the placenta and affects fatty acids and glucose metabolism, promotes lipolysis, and decreases glucose uptake), and insulinase secretion (which is produced by the placenta and facilitates metabolism of insulin). In addition, estrogen and progesterone also contribute to a disruption of the glucose insulin balance. Increased maternal adipose deposition, decreased exercise, and increased caloric intake also contribute to this state of relative glucose intolerance(Amanda , 2008).

1-1-5 Risk Factors for GDM

Several risk factors are associated with the development of GDM. The most common risk factors include a history of macrosomia (birth weight > 4000 g), being a member of an ethnic group with a higher rate of type II diabetes (as mentioned above), polycystic ovarian syndrome, essential hypertension or pregnancy-related hypertension, history of spontaneous abortions and unexplained stillbirths, strong family history of diabetes (especially in first-degree relatives), obesity (pregnancy weight > 110% of ideal body weight or body mass index [BMI] > 30), age older than 25 years, persistent glucosuria, and a history of GDM in a previous pregnancy. No known risk factors are identified in 50% of patients with GDM (Marion , 2008).

1.1.6 Effect of GDM on The Newborn's Health and Risk for Future Diseases

Newborn of women with a history of GDM are also at increased long-term risk of developing metabolic diseases such as obesity, T2DM and the metabolic syndrome. This long-term risk depends on genetic susceptibility and is further modulated by the postnatal environment. In recent years focus has been on the phenomenon of epigenetic transmission of acquired characteristics from mother to child due to

perinatal programming of the fetus. Maternal glucose easily crosses the placenta and as a consequence maternal hyperglycemia leads to intrauterine hyperglycemia, which induces fetalhyperinsulinemia and possible modification of growth and future metabolism of the fetus (fuel-mediated teratogenesis). Also worth noticing, is the finding that the relation between birth weight and risk of T2DM is U-shaped and therefore both infants with decreased and those with increased birth weight are at increased risk of developing T2DM as compared to persons being born with a normal birth weight (Ulla , *et al.*,2015).

1-1-7Association between GDM and Thrombosis in Umbilical Cord Blood

Thrombosis in fetal umbilical cord vessels is a rarely seen condition. It is stated that it is seen at a rate of 1/1300 in the retrospective autopsyresults, 1/1000 in perinatal autopsies, 1/250 in risky gestations and 1/25 only in the cases on which cord anomalies are examined. It was monitored that there was venous thrombosis in 70 % of the Cases determined to have had cord thrombosis, both arterial and venous thrombosis in 20% of them and only arterial thrombosis in 10% of them, the casual mechanisms and related effects of thrombosis are not well understood (Avagliano L, *et al.*, 2010). Maternal diabetes mellitus is risk factor for fetal thrombus formation: infants of diabetic mothers have an increased level of α 2-antiplasmin and decreased fibrinolysin activity with a higher risk of

developing thrombosis. They also have an imbalance between vasodilatation and vasoconstriction factors, with enhanced susceptibility to vasoconstriction and platelet aggregation .In one study aimed to find the coagulation link of the hemostatic system in newborn infants from mothers with GDM, fifty newborn infants born to mothers with extragenital pathology (diabetes mellitus) were studied for the coagulative link of the homeostasis system as were 20 babies born to essentially healthy mothers. The conducted study revealed signs of hypercoagulation presenting with activation of the external and internal route of hemostasis in those infants born to mothers with diabetes mellitus (DM). Toward the period of early neonatal adaptation shifts are still observable in the coagulating system at the expense of stimulation of the first phase of coagulation. The disclosed abnormalities in the coagulative link of the coagulating system in infants born to DM mothers pose a threat of development of thrombohaemorrhagic complications (Znemen'ka, et.al, 2000).

1.1.8 Screening and Diagnosis of GDM

There is a debate regarding the preferred screening protocol for GDM. Some experts recommend universal screening because not all women who develop GDM have risk factors. The ADA policy states that screening may be omitted in low-risk women. A woman is considered

low risk if all of the following factors are present: age younger than 25 years; BMI less than 25 before pregnancy; not of Hispanic, African American, American Indian, South or East Asian, or Pacific Islander descent; no first-degree relative with DM; no history of abnormal glucose tolerance; and no history of poor obstetric outcome. The American College of Obstetricians and Gynecologists (ACOG) practice bulletin states that universal screening is the most sensitive and more practical approach, but it notes that low-risk women may be excluded from screening per the ADA recommendation. The United States Preventive Services Task Force on Preventive Health Care (2008) concluded that there is not enough evidence to support or deny universal screening for GDM.

1.1.8.1 Screening

When the universal screening approach is employed, patients with no known risk factors should undergo a 1-hour glucose test (glucose challenge test) at 24 to 28 weeks of gestation. Patients with known risk factors that indicate the possibility of glucose intolerance may be tested at the onset of prenatal care. If this initial screen is normal, then the test is repeated at the beginning of the third trimester (24 weeks).For the glucose challenge test, the patient receives 50 g of glucose. One hour later, blood is drawn for a plasma glucose determination. A glucose value above 130 to 140 mg/dL is considered abnormal and necessitates a second test, the

3-hour glucose tolerance test. Our center uses 140 mg/dL as the cutoff point. Abnormal results for the 1-hour screening testoccurred in 15% of patients. Of those patients who go on to have the 3-hour screening test, 15% was diagnosed with GDM.To perform glucose tolerance testing (GTT), clinicians first draw a fasting glucose sample and then administer 100 g of glucose. Blood for glucose values is drawn at 1 hour, 2 hours, and 3 hours. Although some centers perform a 75-g 2-hour GTT as both a screening test and a diagnostic test, most centers in the United States rely on the 2-step method described above.

1.1.8.2 Diagnosis

In the Carpenter/Coustan conversion method, diagnosis of GDM is based on the presence of 2 or more of the following factors:

- Fasting serum glucose concentration exceeding 95 mg/dL
- 1-hour serum glucose concentration exceeding 180 mg/dL
- 2-hour serum glucose concentration exceeding 155 mg/dL
- 3-hour serum glucose concentration exceeding 140 mg/dL

Alternatively, some centers employ the National Diabetes Data Group (NDDG) criteria, which are slightly more liberal. The abnormal values are as follows (Metzger BE, 2002):

- Fasting serum glucose concentration exceeding 105 mg/dL

- 1-hour serum glucose concentration exceeding 190 mg/dL
- 2-hour serum glucose concentration exceeding 165 mg/dL
- 3-hour serum glucose concentration exceeding 145 mg/dL

The Hyperglycemic and Adverse Pregnancy Outcomes (HAPO) study showed that increasing levels of plasma glucose are associated with birth weight above the 90th percentile, cord blood serum C-peptide level above the 90th percentile, and, to a lesser degree, primary cesarean deliveries and neonatal hypoglycemia (Crowther CA, *et al* 2005). There were also associations between increased maternal plasma glucose levels and premature delivery, shoulder dystocia, preeclampsia, and hyper-bilirubinemia. The results of the HAPO and Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS)studies indicate that maternal hyperglycemia that does not meet diagnostic criteria for overt diabetes still has a correlation with perinatal disorders and problems. This association suggests a need to reevaluate the standards and criteria for diagnosing and treating hyperglycemia in pregnancy (Gabbe SG, 2003).

1.1.9. Treatment and Management of GDM

Treatment of GDM with diet and insulin reduces health problems mother and child. Treatment of GDM is also accompanied by more inductions of labour.A repeat OGTT should be carried out 6 weeks after delivery, to confirm the diabetes has disappeared. Afterwards, regular screening for

type 2 diabetes is advised. If a diabetic diet or G.I. Diet, exercise, and oral medication are inadequate to control glucose levels, insulin therapy may become necessary. The development of macrosomia can be evaluated during pregnancy by using sonography. Women who use insulin, with a history of stillbirth, or with hypertension are managed like women with overt diabetes (Gabbe SG, Graves CR, 2003).

1.1.9.1 Lifestyle

Counseling before pregnancy (for example, about preventive folic acid supplements) and multidisciplinary management are important for good pregnancy outcomes. Most women can manage their GDM with dietary changes and exercise. Self-monitoring of blood glucose levels can guide therapy. Some women needed antidiabetic drugs, most commonly insulin therapy. Any diet needs to provide sufficient calories for pregnancy, typically 2,000 – 2,500 kcal with the exclusion of simple carbohydrates. The main goal of dietary modifications is to avoid peaks in blood sugar levels. This can be done by spreading carbohydrate intake over meals and snacks throughout the day, and using slow-release carbohydrate sources—known as the G.I. Diet. Since insulin resistance is highest in mornings, breakfast carbohydrates need to be restricted more. Ingesting more fiber in foods with whole grains, or fruit and vegetables can also reduce the risk of gestational diabetes (Gabbe SG, Graves CR, 2003). Regular moderately intense physical exercise is advised, although there is

no consensus on the specific structure of exercise programs for GDM (Gabbe SG, 2003).Self-monitoring can be accomplished using a handheld capillary glucose dosage system. Compliance with these glucometer systems can be low. Target ranges advised by the Australasian Diabetes in Pregnancy Society are as follows (Gabbe SG, Graves CR, 2003).

- Fasting capillary blood glucose levels <5.5 mmol/L
- 1 hour postprandial capillary blood glucose levels <8.0 mmol/L
- 2 hour postprandial blood glucose levels <6.7 mmol/L

Regular blood samples can be used to determine HbA1c levels, which give an idea of glucose control over a longer time period. Research suggests a possible benefit of breastfeeding to reduce the risk of diabetes and related risks for both mother and child (Gabbe SG, Graves CR, 2003).

1.1.9.2 Medication

If monitoring reveals failing control of glucose levels with these measures, or if there is evidence of complications like excessive fetal growth, treatment with insulin might be necessary. This is most commonly fast-acting insulin given just before eating to blunt glucose rises after meals. Care needs to be taken to avoid low blood sugar levels due to excessive insulin. Insulin therapy can be normal or very tight; more injections can result in better control but requires more effort, and

there is no consensus that it has large benefits. A 2016 Cochrane review concluded that quality evidence is not yet available to determine the best blood sugar range for improving health for pregnant women with GDM and their babies.There is some evidence that certain medications by mouth might be safe in pregnancy, or at least, are less dangerous to the developing fetus than poorly controlled diabetes. The medication metformin is better than glyburide. If blood glucose cannot be adequately controlled with a single agent, the combination of metformin and insulin may be better than insulin alone. Another review found good short term safety for both the mother and baby with metformin but unclear long term safety.People may prefer metformin by mouth to insulin injections. Treatment of polycystic ovarian syndrome with metformin during pregnancy has been noted to decrease GDM levels.Almost half of the women did not reach sufficient control with metformin alone and needed supplemental therapy with insulin compared to those treated with insulin alone, they required less insulin, and they gained less weight. With no long-term studies into children of women treated with the drug, there remains a possibility of long-term complications from metformin therapy. Babies born to women treated with metformin have been found to develop less visceral fat, making them less prone to insulin resistance in later life (Gabbe SG and Graves CR, 2003).

1.1.10 Complications of GDM

1.1.10.I Maternal Complications

Women with GDM experience twice the number of urinary tract infections than women who do not have GDM. This increased infection incidence is thought to be due to the increased amount of glucose in the urine beyond the normal glucosuria that is present in pregnancy. There is also an increased risk of pyelonephritis, asymptomatic bacteriuria, and preeclampsia. There is a 10% risk of polyhydramnios that may increase the risk of abruptionplacenta and preterm labor as well as of postpartum uterine atony. Congenital anomalies do not occur at an increased rate in patients with GDM. There is reportedly an increased incidence of stillbirth when glucose control is poor. There is also a 10% per year risk of developing type II diabetes after the pregnancy in which GDM occurred, with the greatest risk within the first 5 years following the index pregnancy (Adam SS, 2009).

1.1.10.2 Neonatal Complications

Macrosomia, if it occurs, typically becomes evident at 26 to 28 weeks gestation. Complications associated with macrosomia include fetopelvic disproportion leading to operative delivery, shoulder dystocia, and neonatal hypoglycemia. There is an increased incidence of hyperbilirubinemia, hypocalcemia, respiratory distress syndrome, and

polycythemia in the Newborn. Long-term complications can include obesity, diabetes during childhood, impaired motor function, and higher rates of inattention and hyperactivity (Adam , 2009).

1.1.11 Management of GDM

The cornerstone of GDM management is glycemic control. The initial treatment for GDM is lifestyle interventions, which include medical nutrition therapy and daily exercise. Patients are required to check their glucose level frequently at home to assure that the glycemic targets are achieved. If the glycemic goals are not accomplished with these measurements, medical therapy should be initiated (Eman,2015).

1.1.11 Blood Glucose Monitoring

Women are instructed to carry out self monitoring of blood glucose 4 times a day, fasting glucose (upon awakening), and one or 2 hour post-meals (after the first bite of a meal). In GDM, monitoring of blood glucose after meals is preferred over pre-meal testing as the risk of macrosomia increases with increased maternal glucose levels post-meals. This was illustrated in a randomized clinical trial, which compared preprandial glucose monitoring to one hour post-prandial testing, and found macrosomia, cesarean deliveries, and neonatal hypoglycemia were significantly less frequent in women who monitor their glucose post-meals. However, it is not known whether a one hour, or 2-hour post-

prandial testing is the ideal goal for the prevention of fetal risks. Therefore, patients can monitor their glucose levels at one or 2 hours post-meal, whatever is convenient, or at the estimated peak blood glucose is most likely to occur post prandial, for example, choosing the time at which glucose was elevated during OGTT (Eman,2015).

1.1.12 Pharmacological Interventions (Insulin Therapy)

If the medical nutrition therapy and exercise fail to achieve glycemic goals for a woman with GDM, insulin therapy should be initiated. The type and timing of insulin should be chosen based on the specific blood glucose elevation. If the fasting glucose is greater than 90-95 mg/dl (whole blood capillary) then basal insulin, long-acting insulin analog, or Neutral Protamine Hagedorn (NPH); 4 units for example, should be started before bedtime. If fasting glucose level is too high, then basal insulin dose can be calculated according to the patient's weight, 0.2units/kg/day. In cases where glucose level is elevated following a meal, rapid-acting insulin, or regular insulin should be prescribed before that specific meal, beginning with 2-4 units, or a dose of one unit per 10-15 g of carbohydrates. If both fasting and PP glucose levels are elevated, a 4-injections-per-day regimen "basal and meal time insulin regimen" should be prescribed.Basal and meal time insulin regimen is preferred over twice dose regimen because it is more likely achieves, maintains target blood glucose, and allows more flexibility.One could start by 2-4

units of rapid-acting insulin, or regular insulin before each meal, and 2-4 units of basal insulin before bed time. Another approach to determine the insulin doses is based on a woman's body weight and gestational week. In the first trimester, the total daily insulin requirement is 0.7units/kg/day, in the second trimester it is 0.8 units/kg/day, and in the third trimester it is 0.9-1.0 units/kg/day. In a morbidly obese woman, the initial doses of insulin may need to be increased to 1.5-2.0 units/kg to overcome the combined IR of pregnancy and obesity. Subsequently, the calculated total daily dose of insulin should be divided into 2 halves; one half given as basal insulin at bed time, and the other half divided between 3 meals, and given as rapid-acting, or regular insulin before meals (Eman,2015).

1.2. Umbilical Cord Blood

Cord blood (umbilical cord blood) is blood that remains in the placenta and in the attached umbilical cord after childbirth. Cord blood is collected because it contains stem cells, which can be used to treat hematopoietic and genetic disorders Figure (1.1) (Young Ho.Lee, 2010).

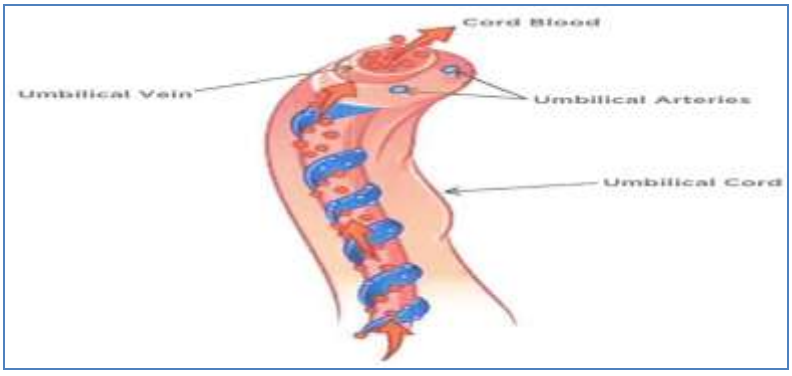


Figure (1.1): Anatomy of Umbilical Cord (Jennifer D. 2007)

1.2.1Umbilical Cord Structure

The umbilical cord at full term, as a rule, is about equal to the length of the foetus, *i.e.*, about 50 cm, but it may be greatly diminished or increased. The umbilical cord is essentially composed of amniotic epithelium covering mucoid mesenchymal connective tissue (Wharton's jelly). Sarugaser*et al* postulated that this connective tissue was derived from a mesenchymal precursor cell population located within the umbilical cord. They reported that these cells were most likely located in close vicinity to the vasculature, thus close to their source of oxygen and nutrients. They called these cells human umbilical cord perivascular (HUCPV) cells (Habibollah S, 2010). The umbilical cord inserts into the chorionic plate of the placenta. It usually has two arteries and a vein, suspended in a hydrated extracellular matrix known as Wharton's jelly. Originally two veins are present, but the second one normally atrophies during the pregnancy. These vessels are developmentally derived from the allantoic vessels. At the junction of umbilical cord and placenta, the umbilical arteries branch radially to form chorionic arteries Figure (1.2). The chorionic arteries further branch before they enter into the villi. In the villi, they form an extensive arteriocapillary venous system, bringing the fetal blood extremely close to the maternal blood; but no intermingling of fetal and maternal blood occurs. This is defined as the placental barrier

and allows selective movement of endogenous and exogenous compounds across the placenta. This protects the developing foetus from the harmful effects of toxins, chemicals, cytokines, micro-organisms etc. The umbilical vessels are responsible for exchange of gases, nutrients and waste products between fetal and maternal circulations across the placental membranes. The umbilical arteries deliver deoxygenated blood to the capillaries of the placental villi where gaseous exchange takes place between maternal and fetal circulations. Oxygenated blood is returned to the fetus via the umbilical vein. Maternal blood in the intervellous spaces is supplied and drained by maternal vessels.

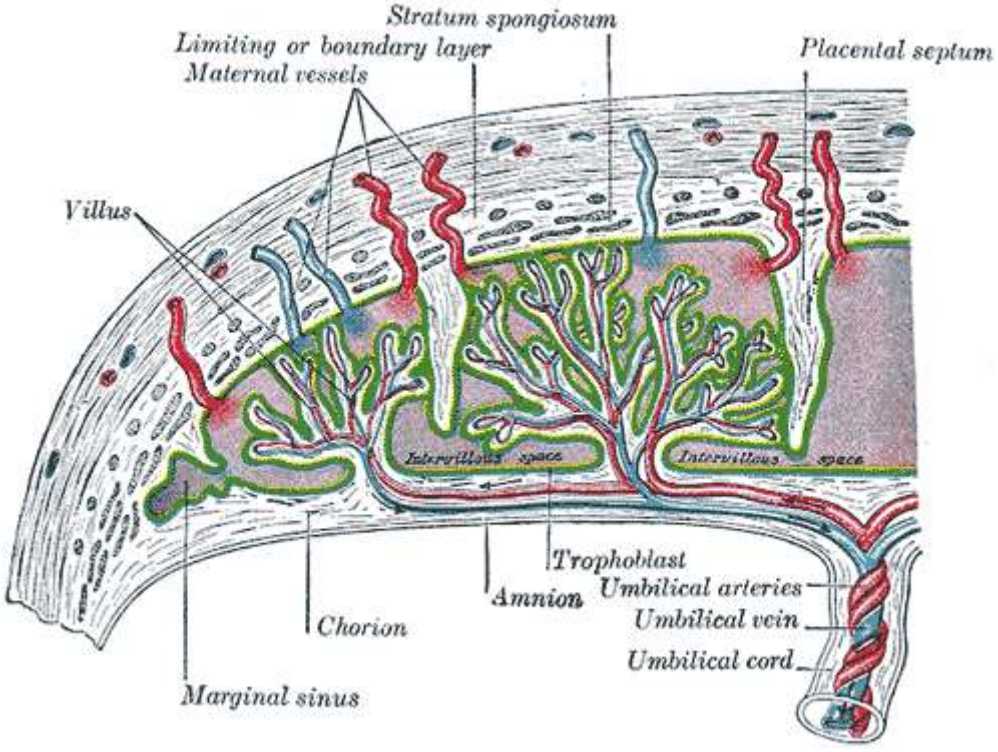


Figure (1.2): Cross section of the placenta (The Bartleby.com edition of Gray's Anatomy of the Human Body)

Human umbilical vessels differ from the major vessels of the same caliber in the body for many reasons. Transudation of fluid occurs in these vessels and contributes to the formation of the amniotic fluid (Blanco, M.V.et.al, 2011).

This blood is usually thrown away. Cord blood contains stem cells, which are special cells that can be used to treat certain diseases in children and adults. These diseases include some cancers (leukemia and lymphoma), blood disorders (sickle cell and thalassemia major), and other life-threatening diseases Human umbilical cord blood (HUCB) contains an average of 150 ml of blood at term and is rich in various components like higher Hemoglobin (20 against 14.4/cmm with 70 percent fetal Hemoglobin), potentiality to carry 60 percent more oxygen than adult Hemoglobin, platelet concentration of 750000 against 250000 per microlitre of adult blood and a WBC count of 24000 against 6500-10,500 cells/μcl in adult blood (Blanco, M.V.*et.al.,* 2011). Hence, studies were started towards utilizing this natural human resource.

1.2.2 Histology of the Umbilical Cords

All umbilical cords included three vessels clearly seen in the stained sections. Infants born to diabetic mothers had thicker umbilical vein intima-media layer and increased both umbilical vein and umbilical artery intima-media areas when compared to the control infants. The umbilical arterial thickness was similar between the groups, Figure (1.3) Umbilical

cord is a fetus-derived organ connecting the fetus to the placenta. It is made of two arteries and a vein surrounded by a few mesenchymal stromal cells and abundant extracellular matrix.The umbilical cord connective tissue has a major role in preventing the vessels from bending and occluding, but the stromal cells are also suggested to participate in the regulation of umbilical cord blood flow together with endothelial cells (Elaine Gluckman, 2015).

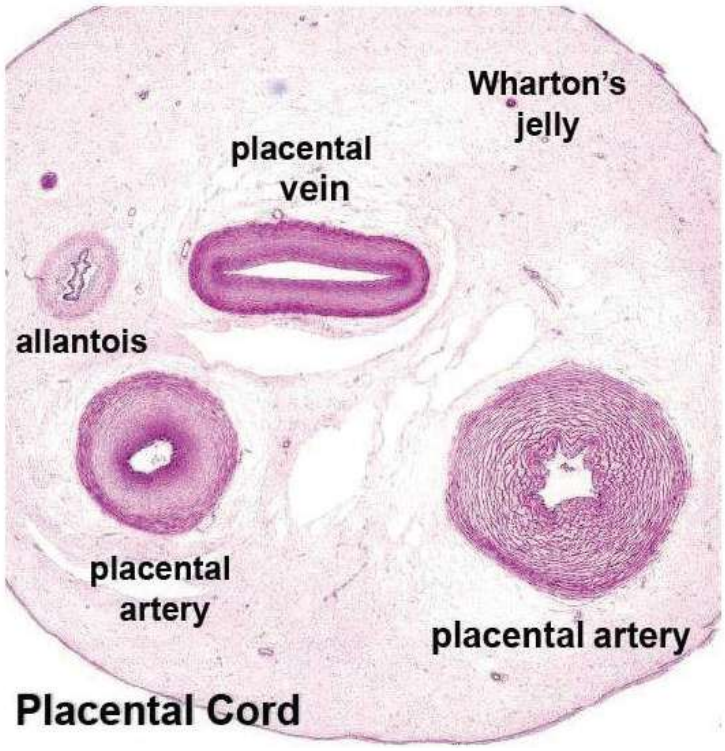


Figure (1.3): A cross-sectional image of a postpartum umbilical cord (Hill M. UNSW2010)

1.2.3 Collection and Storage

Umbilical cord blood is the blood left over in the placenta and in the umbilical cord after the birth of the baby. The cord blood is composed of all the elements found in whole blood. It contains red blood cells, white blood cells, plasma, platelets and is also rich in hematopoietic stem cells.

There are several methods for collecting cord blood. The method most commonly used in clinical practice is the "closed technique", which is similar to standard blood collection techniques. With this method, the technician cannulates the vein of the severed umbilical cord using a needle that is connected to a blood bag, and cord blood flows through the needle into the bag. On average, the closed technique enables collection of about 75 ml of cord blood. Collected cord blood is cryopreserved and then stored in a cord blood bank for future transplantation. Cord blood collection is typically depleted of red blood cells before cryopreservation to ensure high rates of stem cell recovery (Young Ho. Lee, 2010).

1.2.4 Medical Application of Cord Blood

The cord blood (CB) was introduced for the first time in human to reconstitute the hematopoietic system in patient with Fanconi anemia. Since the first cord blood transplantation (CBT), more than 20,000 CBTs have been reported worldwide and more than 400,000 CB units have been stored in more than 100 CB banks. The first report of CBT in Korea was introduced in 1998, since then more than 500 CBTs have been performed and more than 20,000 CB units have been stored for public purposes. Efficacy of unrelated CBT has been demonstrated in children and adults with hematological malignancies and children with a variety of nonmalignant hematologic disorders, including hemoglobinopathies, immunodeficiencies. The clinical use of CB has expanded into various

areas such as treatment of inherited metabolic disorders. Since CB contains hematopoietic stem cells as well as a mixture of multipotent stem cells such as unrestricted somatic stem cells, mesenchymal stem cells, and endothelial colony-forming cells, CB has the ability to regenerate numerous tissue types with functional improvements. Recently, the use of CB in several regenerative medicine applications has expanded its clinical utility. The application of CB for regenerative medicine is different from typical hematopoietic stem cell transplantation (HSCT) which has been performed for inherited metabolic disorders (IMD) requiring pre-conditioning chemotherapy regimens Figure (1.4)(Young-Ho. Lee, 2010).

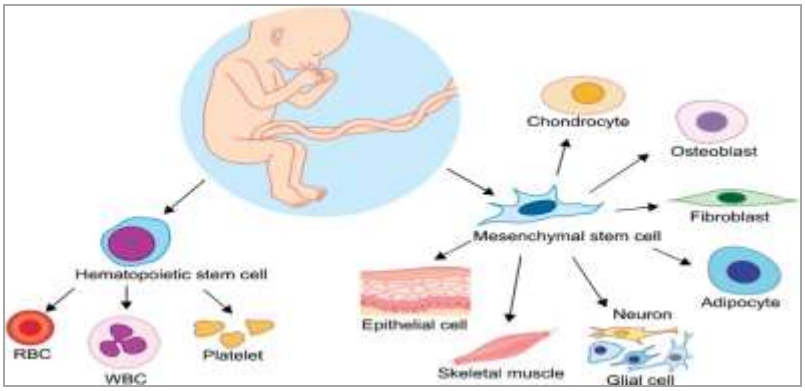


Figure (1.4): Cord Blood Cells Types (Young-Ho. Lee, 2010)

Cord blood is used the same way that hematopoietic stem cell transplantation is used to reconstitute bone marrow following radiation treatment for various blood cancers, and for various forms of anemia. Its efficacy is similar as well (Young Ho. Lee, 2010).

Table 1.1: Clinical Uses of Umbilical Cord Blood to Date (Young Ho. Lee, 2010).

- Infantile Krabbe's disease
- Epstein-Barr virus
- Lysosomal and peroxisomal storage diseases
- Diamond-Blackfan anemia
- Various acute and chronic leukemias
- Neuroblastoma
- Non-Hodgkin's lymphoma
- Hodgkin's lymphoma
- Sickle-cell anemia
- Cooley's anemia
- Hurler syndrome
- Leukocyte adhesion deficiency
- Evans syndrome
- Osteopetrosis
- Spinal cord injury

Successes Using of Umbilical Cord Blood to Treat a Variety of Disease and Injury included (Young Ho. Lee, 2010):

- **Acute Leukemia**
- **Chronic Leukemia**
- **Myelodysplastic Syndromes**

- **Lymphomas**
- Anemias:
    - Aplastic Anemia
    - Congenital Dyserythropoietic Anemia
    - Fanconi Anemia *(Note: the first cord blood transplant in 1988 was for FA, an inherited disorder)*
    - Paroxysmal Nocturnal Hemoglobinuria *(PNH)*
    - Pure Red Cell Aplasia
- **Inherited Red Cell Abnormalities**:
    - Beta Thalassemia Major (*also known as Cooley's Anemia*)
    - Blackfan-Diamond Anemia
    - Pure Red Cell Aplasia
    - Sickle Cell Disease
- **Inherited Platelet Abnormalities**:
    - Amegakaryocytosis / Congenital Thrombocytopenia
    - Glanzmann Thrombasthenia
- **Inherited Immune System Disorders - Severe Combined Immunodeficiency** *(SCID)*:
    - SCID with Adenosine Deaminase Deficiency *(ADA-SCID)*
    - SCID which is X-linked
    - SCID with absence of T & B Cells
    - SCID with absence of T Cells, Normal B Cells
    - Omenn Syndrome

- **Inherited Immune System Disorders – Neutropenias**
- **Myeloproliferative Disorders**
- **Phagocyte Disorders**
- **Leukodystrophy Disorders**
- **Lysosomal Storage Diseases**

1-2-5 Normal Range of Umbilical Cord Blood Parameters

1-2-5-1 Hematological Parameters

In Parallel with Beckman Coulter Ac T5diff Normal Adult Values, and the Normal Range of Values (Based on Dacie and Lewis3) adopted by (Katsares, *et al.*, 2015)(Table 1.2).

Table (1.2) Normal Range of Hematological Parameter (Katsares, *et al* (2015)

1.1.4 Hematological Parameters in Umbilical Cord	European Values (Range, Mean ± SD) (Male and Female)	Beckman Coulter Ac•T 5diff (Normal Adult Values)	Mean ± SD (Male and Female)
WBC (103/µL)	4.0–11.00	4.0–11.0	7.2 ± 3.4 7.1 ± 3.4 (M)*
RBC (106/µL)	4.5–5.5 5.0 ± 0.5(M) 3.8–4.8 4.3 ± 0.5 (F)	4.00–6.20	2.46 ± 0.82
HGB (g/dL)	13–17 15 ± 2 (M) 12–15 13.5 ± 1.5 (F)	11.0–18.8	8.8 ± 2.9

HCT (%)	40–50 45 ± 5 (M) 36–4.6 41 ± 5 (F)	35.0–55.0	25.9 ± 8.8
MCV (fL)	83–101 92 ± 9 (M, F)	80–100	105 ± 6
MCH (pg)	27.0–32.0 29.5 ± 2.5 (M, F)	26.0–34.0	35.8 ± 3.1
MCHC (g/dL)	31.5–34.5 33.0 ± 1.5 (M, F)	31.0–35.0	34.3 ± 7.3
RDW (%)	11.6–14.0 12.8 ± 1.2	10.0–20.0	12.1 ± 1.6 12.3 ± 1.7(M)* 12.0 ± 1.6 (F)*
PLT (103/µL)	150–400	150–400	160 ± 59 157 ± 58 (M)* 164 ± 60 (F)*
MPV (fL)	NA	6.0–10.0	8.0 ± 0.7
NE# (103/µL)	2–7	2.00–8.00	3.4 ± 1.8 3.2 ± 1.7 (M)* 3.6 ± 1.9 (F)*
Ly# (103/µL)	1.00–3.00	1.00–5.00	2.66 ± 1.29

1-2-5-2Coagulation Parameters

Normal values coagulation parameters in the newborn adopted by (Lippi, *et al*., 2007) Table (1.3).

Table (1.3) Normal Range of Coagulation Parameter (Lippi, *et al* 2007)

Coagulation Parameter	**Normal Range**
Prothrombin time (sec)	11-15
Partial thromboplastin time (sec	30-40
Fibrinogen (mg/dl)	175-350
Fibrin split products (mcg/ml	<10
Thrombin time (sec)	15-20

1.3 Homeostasis

Homeostasis is defined as arrest of bleeding, comes from Greek, haeme meaning blood and stasis meaning to stop (Thornton P and Douglas J, 2010).The normal haemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors .An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The haemostatic system thus

represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process for fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels (Hoffbrand, *et al*., 2011)**.** German pathologist Rudolph Virchow described thrombi and their tendency to embolize. He proposed that the formation of thrombi is predisposed by abnormalities in blood flow, vessel wall and blood components. This lead to the formation of 'Virchow's triad, demonstrated in Figure(1.5) for thrombogenesis. It was a simplified view of thrombosis formation predisposition. Now it is known that the process of thrombus formation requires complex interactions involving injury to the vascular endothelium, platelet adherence, aggregation and release and clotting factor activation, eventually leading to thrombin generation and fibrin formation (Wolberg AS, 2012).

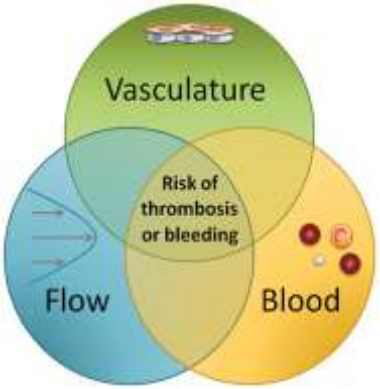


Figure (1.5): Virchow's triad(Wolberg AS, 2012)

1.3.1 Primary Homeostasis

Primary homeostasis results from complex interactions between platelets, vessel wall and adhesive proteins leading to the formation of initial platelet plug. (Palta , *et al*., 2014).

1.2.3 Secondary Homeostasis

Secondary homeostasis consists of the cascade of coagulation serine proteases (Table: 1.1) that culminates in cleavage of soluble fibrinogen by thrombin, Thrombin cleavage generates insoluble fibrin that forms a crosslinked fibrin mesh at the site of an injury. Fibrin generation occurs simultaneously to platelet aggregation (Furie B .2009). It has been traditionally classified into intrinsic and extrinsic pathways, both of which converge on factor X activation Figure(1.8). The majority of coagulation factors were discovered in the middle of the 20^{th} century when doctors and scientists were presented with patients with different coagulation pathologies (Gale AJ 2011 - Palta , Saroa R,2014). While most factors are also named after either the person who discovered them or the patient in whom it was first discovered they have also been given roman numerals. As it was also mentioned previously these numbers were given according to time of discovery and not according to their turn in the coagulation pathway (Vest, 2017). Coagulation factors are inactivated enzymes or precursor proteins (also called zymogens) that circulate in the blood and become activated when a blood vessel is injured. The activation results in

a step-wise amplification of the response. This step-wise activation of coagulation factors is called the coagulation cascade. The coagulation cascade consists of intrinsic pathway and extrinsic pathway, these two pathways join together in the common pathway, seen in Figure (1.6). The first part of the coagulation cascade is differentiated by how it is initiated and the factors involved. The intrinsic pathway is initiated by factors present in the blood while the extrinsic pathway Isis initiated when its components come into contact with tissue factor (TF) located outside the vascular system . The extrinsic pathway is initiated when FVII comes into contact with TF. TF becomes exposed to blood components during injury to endothelium. It activates FVII to FVIIa. TF together with FVIIa then activates FX to its active form FXa. The intrinsic pathway works similarly to the extrinsic pathway. It is initiated when the plasma protein known as Hageman factor (FXII) comes into contact with a negatively charged surface, eg activated platelets. FXII becomes FXIIa. FXIIa together with high molecular weight kininogen (HMWK) activates FXI to FXIa. FXIa, together with FXa and thrombin cleave FVIII to FVIIIa, which acts as a cofactor in the next step. FIXa and FVIIIa, together with calcium and negatively charged phospholipids convert FX to FXa. FXa arises from both the intrinsic and the extrinsic pathway, it is of no importance from where it is activated, the coagulation cascade will continue along the common pathway. The common pathway begins with

FXa binding to cofactor FV, and in the presence of calcium this complex forms the prothrombinase complex. This complex converts prothrombin into thrombin, which in turn converts fibrinogen to fibrin and generates the fibrin clot. The last phase of hemostasis is fibrinolysis. This involves the dissolving of the blood clots during the process of wound healing. It also prevents the formation of clots in healthy blood vessels. The fibrinolytic system is initiated simultaneous with the activation of the coagulation cascade to limit the size of the clot. It is an enzymatic process where plasmin dissolves the fibrin clot into fibrin degradation products. Plasmin, generated from plasminogen by the proteases tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Plasmin activity is in turn regulated by its inhibitor, alpha-2-antiplasmin, to prevent widespread fibrinolysis (Leung LLK, 2016).

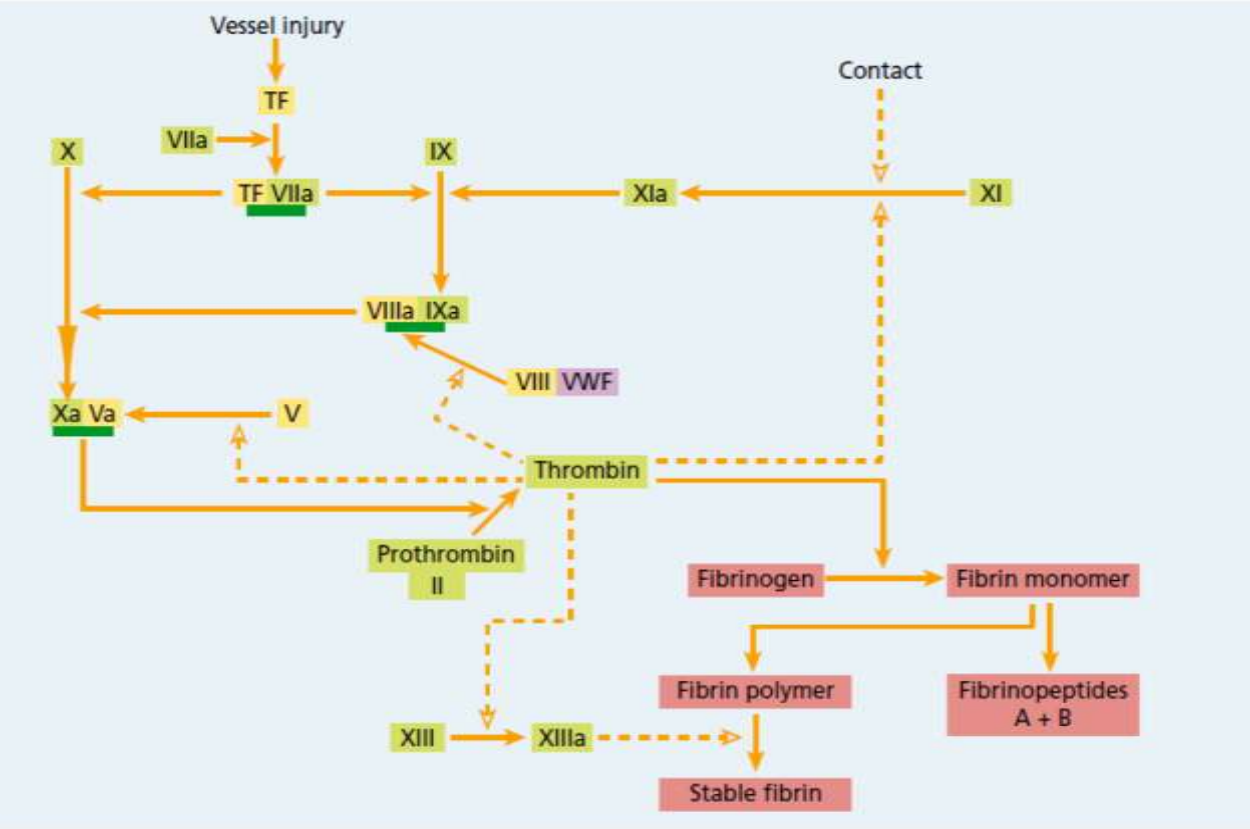
Table 1.4 : Nomenclature of the coagulation proteins/clotting factors (Palta S, *etal*. 2010)

Clotting factor number	Clotting factor name	Function	Plasma half-life (h)	Plasma concentration (mg/L)
I	Fibrinogen	Clot formation	90	3000
II	Prothrombin	Activation of I, V, VII, VIII, XI, XIII, protein C, platelets	65	100
III	TF	Co factor of VIIa	-	-
IV	Calcium	Facilitates coagulation factor binding to phospholipids	-	-
V	Proacclerin, labile factor	Co-factor of X-prothrombinase complex	15	10
VI	Unassigned			
VII	Stable factor, proconvertin	Activates factors IX, X	5	0.5
VIII	Antihaemophilic factor A	Co-factor of IX-tenase complex	10	0.1
IX	Antihaemophilic factor B or Christmas factor	Activates X. Forms tenase complex with factor VIII	25	5
X	Stuart-Prower factor	Prothrombinase complex with factor V. Activates factor II	40	10
XI	Plasma thromboplastin antecedent	Activates factor IX	45	5
XII	Hageman factor	Activates factor XI, VII and prekallikrein		-
XIII	Fibrin-stabilising factor	Crosslinks fibrin	200	30
XIV	Prekallikerin (F Fletcher)	Serine protease zymogen	35	
XV	HMWK- (F Fitzgerald)	Co factor	150	
XVI	vWf	Binds to VIII, mediates platelet adhesion	12	10 µg/mL
XVII	Antithrombin III	Inhibits IIa, Xa, and other proteases	72	0.15-0.2 mg/mL
XVIII	Heparin cofactor II	Inhibits IIa	60	-
XIX	Protein C	Inactivates Va and VIIIa	0.4	-
XX	Protein S	Cofactor for activated protein C		-

HMWK = High molecular weight kininogen; vWf = Von Willebrand factor; TF = Tissue factor

1.3.2.1 Extrinsic Pathway:

It is considered as the first step in plasma mediated haemostasis. It is activated by TF, which is expressed in the subendothelial tissue. Under normal physiological conditions, normal vascular endothelium minimizes contact between TF and plasma procoagulants, but vascular insult expose TF which binds with factor VIIa and calcium to promote the conversion of factor X to Xa (Owens AP 3rd and Mackman N, 2010).



Figure(1.6):The pathway of blood coagulation cascade. (A.V. Hoff brand and P.A.H. Moss. 2011)

1.3.2.2 Intrinsic Pathway

It is a parallel pathway for thrombin activation by factor XII. It begins with factor XII, HMW kininogen, prekallekerin and factor XI (contact family) which results in activation of factor XI. Activated factor XI further activates factor IX, which then acts with its cofactor (factor VIII) to form tenase complex on a phospholipid surface to activate factor X (Hall JE, 2010).

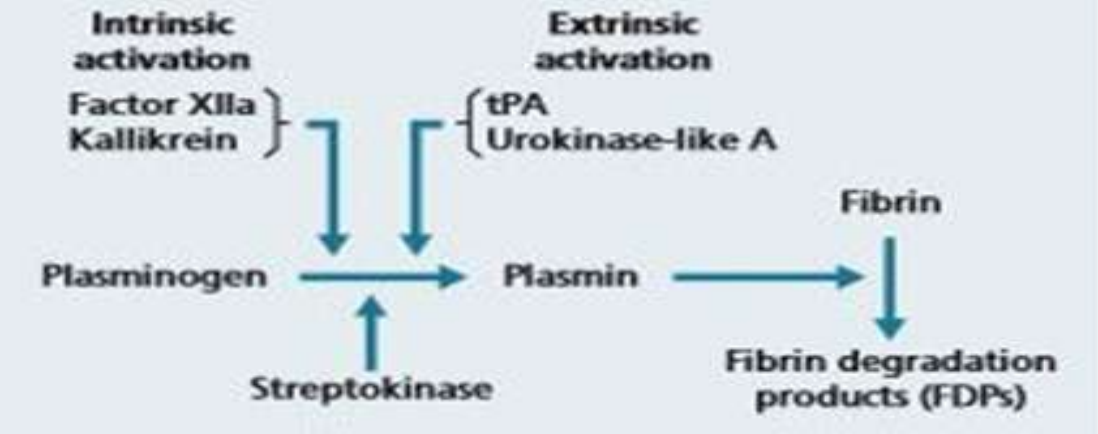
1.3.2.3 Common Pathway

Activated factor X along with its cofactor (factor V),tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts prothrombin to thrombin. This thrombin further cleaves circulating fibrinogen to insoluble fibrin and activates factor XIII, which covalently crosslinks fibrin polymers incorporated in the platelet plug. This creates a fibrin network which stabilises the clot and forms a definitive secondary haemostatic plug (Kumar V, *et al*., 2010).

1.3.2.4 Fibrinolytic System

Fibrinolytic system is a parallel system which is activated along with activation of coagulation cascade and serves to limit the size of clot. Fibrinolysis is an enzymatic process that dissolves the fibrin clot into fibrin degradation products (FDPs) by plasmin originating from fibrin

bound plasminogen in liver. This reaction is catalyzed by tPA or urokinase plasminogen activator (u-PA) released from vascular endothelium. The release of t-PA is stimulated by tissue occlusion, thrombin, epinephrine, vasopressin and strenuous exerciseFigure (1.7).



Figure(1.7): The Fibrinolytic System(A.V. Hoff brand and P.A.H. Moss. 2011)

Plasmin activity is tightly regulated by its inhibitor (α-2 antiplasmin) thus preventing widespread fibrinolysis. In vivo activity of the fibrinolytic system is assessed clinically by measuring the FDP's. D-dimers are produced by digestion of cross linked fibrin and are specific indicators of fibrinolysis used in the assessment and diagnosis of pulmonary embolism, DIC or deep vein thrombosis Since plasmin has the potential to degrade fibrinogen leading to deleterious consequences, the fibrinolytic activity is limited by following factors:

- **Plasminogen activator inhibitor**: the mainphysiological inhibitor of fibrinolysis and acts by inhibiting t-PA and u-PA irreversibly.

- **TAFI thrombin activatable fibrinolysis inhibitor**: It is a plasma proenzyme synthesized by liver and activated by thrombin. It decreases the affinity of plasminogen to fibrin and augments the action of anti-trypsin in inhibiting plasmin.

- **Plasmin inhibitors**: α2 antiplasminand α2Macroglobulin are the glycoproteins that exert action by virtue of plasmin inhibition (Ejiofor JA. 2013).

1.3.3 Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT):

PT and APTT are by far the most common screening tests for coagulation abnormalities. These tests correspond respectively to extrinsic and intrinsic pathways of the Waterfall/Cascade model (Fig. 2A). PT was originally developed by Quick for measuring the prothrombin level by adding a large amount of TF (rabbit brain extract) to plasma. It is now understood that PT is affected by reductions of Factors VII, X, V, and prothrombin such as occur with vitamin K antagonis therapy or severe liver disease. In the PT assay, the amount of TF used to trigger *in vitro* clotting is in large excess compared with *in vivo* conditions, leading to rapid generation of thrombin, and its feedback activation of Factor V. However, it is evident that the *in vivo* coagulation mechanism is not fully reflected by PT because recurrent bleeding occurs in hemophilia (Factor

VIII or Factor IX deficiency) despite normal PT values. The concentration of TF is presumably much lower *in vivo,* and thus, PT was modified for evaluating hemophilic plasma using “partial thromboplastin” (i.e., phospholipid with minimal TF isolated from crude thromboplastin by ultracentrifugation and dilution). In the PTT (partial thromboplastin time) test, fVIIa-mediated fXa and thrombin productions are limited under the condition of low TF, and, as a result, the activities of fIXa and fVIIIa as an alternative source of fXa become critical for clotting . The APTT was further improving for reproducibility by adding a contact activator (e.g., kaolin, celite, or ellagic acid) in an assay known as the activated PTT (APTT). In the presenc of a contact system activator, a series of serine protease activations occur in the descending order of Factor XIIa 3 XIa 3 IXa 3 Xa, resulting in thrombin generation. Although Factor XII activation bya contact activator is not considered important fornormal hemostasis (because Factor XII deficient patientsdo not bleed), APTT is sensitive to gross reductionof Factors XII, XI, IX, VIII, V, and to a lesserextent, prothrombin. The sequence of serineprotease activations proceeds very slowly in APTT because the cofactors, fVIIIa and fVa, are not availableuntil thrombin is generated to activate them. Thus, APTT is used clinically for monitoring of unfractionated heparin, argatroban, bivalirudin, and lepirudin anticoagulation (note: a specific calibration isrequired for each anticoagulant), because all these thrombin inhibitors

reduce thrombin-mediated feedback activation of Factors VIII and V. Although PT/APTT can be used to guide anticoagulation several important limitations should be notedwhen they are being measured to evaluate bleeding. Perioperatively, bleeding is caused by multiple coagulationdefects because of hemodilution, consumptiveloss, fibrinolysis, anticoagulant use, hypothermia, andother mechanical and metabolic derangement. Importantly, PT/APTTdo not provides any informationon *in vivo* interaction of platelets with coagulationfactors. Activated platelets are capable of locally accumulatingcoagulation factors, and thus, the extent of bleeding under prolonged PT/APTT may vary accordingto the platelet count and/or function. Further, it isnot possible to estimate the overall stability of a hemostatic thrombus using PT/APTT because bothtests are terminated before fibrin is polymerized by fXIIIa. Congenital Factor XIII deficiency is associated with umbilical cord bleeding and intracranial hemorrhage, but this deficiency is not detected by PT/APTT screening. PT/APTT also remain normal when bleedingis caused by increased fibrin breakdown (i.e., hyperfibrinolyticstate) such as occurs in congenital deficiency of _2-antiplasmin.31 In contrast to PT/APTT, the use of thrombelastography/metry allow functional activities of fibrinogen, Factor XIII, and fibrinolytic proteins (Tanaka, K.A., Key, N.S.2009).

1.3.4 International Normalized Ratio

The result (in seconds) for a prothrombin time performed on a normal individual will vary according to the type of analytical system employed. This is due to the variations between different types and batches of manufacturer's tissue factor used in the reagent to perform the test. The INR was devised to standardize the results. Each manufacturer assigns an ISI value (International Sensitivity Index) for any tissue factor they manufacture. The ISI value indicates how a particular batch of tissue factor compares to an international reference tissue factor. The ISI is usually between 0.94 and 1.4 for more sensitive and 2.0-3.0 for less sensitive thromboplastins. The INR is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the ISI value for the analytical system being used.

1.3.5 Cord Blood Coagulation Factors Relationship

The homeostasis of healthy newborn differs from those of normal adult but remains well balanced without bleeding or thrombosis. However, this equilibrium is unstable, and the neonate is exposed to acquired or inherited homeostasis disorders that necessitate to be early diagnosed in order to be appropriately treated.The newborn is at risk for vitamin K deficiency with bleeding due to poor transport of vitamin K across the placenta and low levels of coagulation factors(Gruel Y,2010).

Coagulation factors do not cross the placental barrier but are synthesized independently by the conceptus. At birth, activities of the vitamin K dependent factors II, VII, IX, and X and the concentrations of the contact factors XI and XII are reduced to about 50% of normal adult values. The levels of the factors V, VIII, XIII, and fibrinogen are similar to adult values. Plasma concentrations of the naturally occurring anticoagulant proteins (antithrombin, protein C, and protein S) are significantly lower at birth than during the adult years. The diagnosis of some inherited coagulation deficiencies can be difficult in the newborn due to physiologically low levels of coagulation factors (Pichler*et al*,2008)**.**

However, there were studied in coagulation factors, which measure fibrinogen level in newborn cord blood between GDM and normal pregnancy. This physiological mechanism may convert into a pathologic process in a pregnancy complicated by GDM. Since the coagulation cascade and the fibrinolytic system involve various coagulation factors interacting through complex pathways, it becomes difficult to reveal and even understand the underlying mechanisms of the hemostatic changes occurring in the glucose metabolism. Considering the impact of GDM on the coagulation system, the dynamics involved at a pathophysiological level and the exact mechanism remain still unclear (LemkesBA*et al*., 2010).

1.4 Previous Studies

Neary*et al* (2015) conducted a study in Rotunda Hospital, Dublin, Ireland,One hundred and sixteen infants were recruited.Cord and peripheral blood of neonates < 30 weeks gestational age (GA) was drawn at birth, on days 1 and 3 and fortnightly until 30 weeks corrected gestational age. The following parameters were studied - Prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and coagulation factor levels.There were prolongation of PT, APTT, and in significant increase in plasma fibrinogen between the two groups was observed (*P.value*< 0.05).

Christensen *et al* (2014) a study of coagulation tests at 175 preterm deliveriesfrom the umbilical vein near the placenta. Fibrinogen, prothrombin time, activated partial thromboplastin time, D-dimer, platelet (PLT) count, and mean PLT volume were measured. There were no abnormal coagulation value.

Datonye, *et al,* (2007) at Nigerian Newborn, the study involving 60 subjects consisting of 30 newborn neonates and their respective mothers. Haematocrit, erythrocyte sedimentation rate, haemoglobin concentration, red blood cell count, white blood cell count, whole blood relative viscosity, relative plasma viscosity, fibrinogen concentration, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were determined.There was

significantly lower value of fibrinogen found in umbilical cord blood compared to maternal blood (*P.value< 0.05*).

Lippi G, *et al* (2007) a study the postnatal development of the human coagulation system in newborn infants and to develop appropriate reference ranges for prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen. The mean FBG value was within the adult reference range in newborns at birth.

In 2000 Znamens'ka TK and Zhdanovych OI done a study include fifty newborn infants born to mothers with extragenital pathology (diabetes mellitus) were studied for the coagulative link of the homeostasis system as were 20 babies born to essentially healthy mothers. The studies were made by day 1 to 3 and 5 to 7 the babies' life. The conducted studies revealed signs of hypercoagulation presenting with activation of the external and internal route of homeostasis in those infants born to mothers with diabetes mellitus (DM). Toward the period of early neonatal adaptation shifts are still observable in the coagulating system at the expense of stimulation of the first phase of coagulation. The disclosed abnormalities in the coagulative link of the coagulating system in infants born to DM mothers pose a threat of development of thrombohaemorrhagic complications.

1.5 Rationale

Contact of diabetes mellitus (DM) on the coagulation system and endothelial functions is known for many years. Homeostatic factors and activities are influenced both by the hyperglycemic state and hypoglycemic in DM. Newborns of women with a history of GDM or risky gestation are also at increased long-term risk of developing metabolic diseases and macrosomia. Maternal glucose easily crosses the placenta and as a consequence maternal hyperglycemia leads to intrauterine hyperglycemia, which induces fetal hyperinsulinemia and possible modification of growth and future metabolism of the fetus. The association between hyperglycemia and thrombosis is well known, and thrombosis in fetal umbilical cord vessels rate of 1/250 in risky gestations. It was monitored that there was venous thrombosis in 70 % of the cases determined to have had cord thrombosis, the casual mechanisms and related effects of thrombosis are not well understood. There is an increased prothrombotic state due to increased activation of platelets and prothrombotic coagulation factors coupled with a decrease in fibrinolysis. Pregnancy is a hypercoaguble state in itself. However,this physiological mechanism may convert into pathologic process in a pregnancy complicated by GDM. Thrombosis tends to occur in neonatal of diabetic mothers(Edstrom and Christensen,2000).So, there is evidence that the risk of thrombosis is increased. Determining an affordable biomarker that

can predict the effect of GDM on cord blood would provide a better outcome for newborn.Therefore, this research is intended to open new avenues toward PT and APTT assays as an initial evaluation and clinical utility in the prediction of effects of GDM on neonatal cord blood. This study aimed to assess PT and APTT in cord blood of newborn in mothers with gestational diabetes mellitus. Studies of the effect of gestational diabetes in Sudan are not yet conducted so this study take place as starter for coming studies to verify the relation between the gestational diabetes and the thrombosis.

1.6Objectives

1.6.1 General Objective

Evaluation of PT and APTT in newborn cord blood samples among mothers with gestational diabetes mellitus.

1.6.2 Specific Objectives

- To estimate PT and APTT in newborn cord blood of healthy mothers.

- To compare the PT and APTT in newborn cord blood of gestational diabetes mellitus mothers and newborn cord blood of healthy mothers.

- To compare the cord blood PT and APTT according to different FBG levels among mothers.

- To correlate PT and APTT in the cord blood with age, history of DM and BMI of GDM women.

- To correlate PT and APTT between newborns cord blood gestational diabetes mellitus mothers and their gestational weeks and weight of newborns.

Chapter Two

2. Materials and Methods

2.1 Materials

A cross sectional comparative study conducted at the faculty of Medical laboratory science, Sudan University of Science and Technology from February to August 2018.Sixty(60) Newborns from 30 mothers with GDM and 30 healthy mothers were enrolled in this study after ethical consent and hospital approval. Citrate cord blood samples were performed for fibrinogen estimation.The practical work and newborns selection were performed at Saad Aboulela University Hospital and, Al-Qma Specialized Hospital during and, Alsaaha Specialized Hospital, Khartoum, Sudan.

2.1.1 Inclusion criteria

- Gestational diabetes mellitus pregnant women.
- Healthy pregnant women (as control).

2.1.2 Exclusion criteria

- Newborn to mothers with liver dysfunction
- Newborn to motherswith renal dysfunction
- Newborn to motherswith preeclampsia/eclampsia
- Newborn to motherswith endocrine disorders

- Use of medications with known effects on haemostatic system such as aspirin, heparin, and enoxaparin.
- Prematurely born newborns.

2.1.3 Ethical Consideration

Ethical committee of research in the Faculty of Medical Laboratory Science was approved the study. The purpose and objectives of the study was explained to each one of participants, the participant has right to voluntary informed consent, has right to withdraw at any time without any deprivation, assured them that the data collected will remain confidential and it's not allowed for any person to identify it. The questionnaire was filled in their rest time, and participant has right to benefit from the researcher knowledge and skills. Samples were coded and confidentiality of patient data was maintained throughout the study by locking hard copies and password protecting electronic files.

2.2 Methods

2.2.1 Sample Collection

Volunteering pregnant women after getting an informed consentand before delivery were clinically examined by the doctors whether she is gestational diabetes mellitus or normal. 2.5 ml of new born umbilical cord blood was collected in plastic container containing 3.2% ml tri sodium citrate as anticoagulant, and then the blood is centrifuged, after

thoroughly mixing, for 15 minutes at 3000 rpm to obtain platelets poor plasma (PPP).

2.2.2 Prothrombin time (PT) using automated CL– 2000 Biobase Coagulometer

2.2.2.1 Principle

The prothrombin test measures the clotting time of the plasma in the presence of an optimal concentration of tissue extraction (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system.

2.2.2.2 Procedure

PT liquid thromboplastin brought to 37c and was well mixed, coagulometer was adjusted at 37c and cuvettes with magnetic stirrers inside were kept in holes at 37c for 2 minutes then 200ml of prothrombin liquid thromboplastin added to the plasma and simultaneously was measured at the end point, the clotting time determined directly from the display.

2.2.2.3 Component and Reagent

1. Pooled normal control plasma.

2.Thromboplastin reagent which contains tissue factor and phospholipid obtained from human or rabbet brain or recompenant DNA.

2.2.2.4 Normal Values

The normal range of PT is between 11-16 seconds.

2.2.2.5 Interpretation of the Results

The common cause of prolonged PT is:Administration of oral anticoagulant drugs (vit k antagonists), liver disease, vit k deficiency, DIC, or prothrombin deficiency or defect.

2.2.3 Activated partial thromboplastin time (APTT) using automated CL – 2000 Biobase

2.2.3.1 Principle

The test measures the clotting time of plasma often the activation of contact factors but without added tissue thromboplastin, and so indicates the overall efficiency of intrinsic pathway. To standardized the activation of contact factors. The plasma is first pre-incubated with kaolin. Standardaized phospholipid is provided to allow the test to be performed on PPP. The test depends at only the contact factor and on factors VII and IX , but also on the reactions with factor X,V,II,I, it is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin.

2.2.3.2 Procedure

Activated partial thromboplastin reagent was brought to 37c and was well mixed, coagulometer was adjusted at 37c and cuvettes with magnetic stirrer inside were kept in holes at 37c , 100 ml of plasmas placed in cuvettes, 100ml of APTT reagent which was pre-warmed at 37c was added to plasma , mixed well and incubate for 3 minutes , 100ml of Cacl2 which was pre-formed at 37c was added and simultaneously the display

started to measure the time , then the time of clot formation was observed directly from display.

2.2.3.3 Component of the Reagent

1. Polled normal control plasma.

2. Cephalin reagent, Cacl2, 0.025 %water bath

2.2.3.4 Normal Range

The normal range is typically within 26-40 seconds.

2.2.3.5 Interpretation of the Results

The common cause of prolonged APTT is: DIC, liver disease, massive transfusion with plasma deplete, administration of or contamination with heparin or other anticoagulants red blood cell, a circulating anticoagulant (inhibitor) and deficiency of coagulation factor other than factor (VII).

2.2.4 Data analysis

The collected data proceed for analysis using SPSS version 20 computeri -zed program and the data presented in form of tables and figures.

Chapter Three

3. Results

3.1 Demographic Data of Pregnant Women

A total of 60 Sudanese volunteer with gestational diabetic pregnant women and matched healthy pregnant women as control were enrolled in this study; 73.3% of gestational diabetic pregnant women aged<35 years whereas 26.7% of them were >35 Years. Also 53.3% of them had history of diabetes mellitus whereas 46.7% were not. On other hand 37% of them had family history of DM whereas 63% were not. On the other hand, 83% healthy pregnant women <35 years whereas 17% of them >35 Years Moreover, the results showed significant increase in BMI, FBG level and newborn weight among GDM mother *(P.value <0.05)*Table (3.1).

Table: (3.1) Age and Clinical Data Frequencies

Variables	**Case**		**Control**	
Age	**Frequency**	**(%)**	**Frequency**	**(%)**
<35 Years	22	73.3	25	83
>35 Years	8	26.7	5	17
History of DM				
Yes	16	53.3		
No	14	46.7		

FH of DM				
No	19	63.0		
DM	11	37.0		
BMI				
<25	10	33.0	19	63
>25	20	67.0	11	37
Total	**30**	**100**	**30**	**100**

3.2 Descriptive Analysis

3.2.1 The age groups

The Mean of GDM Patients who were hypercoagulable was significant increase in the age > 35 years (22.26±3.99) more than Age < 35 years (18.65±4.31) according to PT. It's also the same in INR result which was significant increase in the age > 35 years (1.86±0.41) more than Age < 35 years (1.55±0.44). Otherwise the mean of APTTin newborns cord blood of GDM was significant increase in the age < 35 years (53.72±3.89) to the age > 35 years (53.01±3.94). Its show that there was a significant correlation between age and hypercoagulation (Table 3.2).

Table (3.2) shows the level of age in PT,INR and APTT among patients

Parameters	<35 Years(Mean±SD)	>35Years(Mean±SD	*P-value*
PT	18.65±4.31	22.26±3.99	0.044
INR	1.55±0.44	1.86±0.41	0.097
APTT	53.72±3.89	53.01±3.94	0.663

3.2.2 History of Diabetes

A significant increase in the Mean of GDM Patients that have history of diabetes (21.02±4.53) to the GDM Patientsthathaven't history of diabetes (18.00±3.94) , While there's No significant in the Mean of INR of GDM Patients that have history of diabetes (1.75±0.45) to the Patients that havn't history of diabetes (1.50±0.42). Also there's No significant in the Mean of APTT of GDM Patients that have history of diabetes (54.14±3.46) to the Patients that haven't history of diabetes (52.84±4.28), as shown in (Table 3.3).

Table (3.3) shows the association of parameters with history of disease

Parameters	Yes (Mean±SD)	No (Mean±SD)	*P-value*
PT	21.02±4.53	18.00±3.94	0.061
INR	1.75±0.45	1.50±0.42	0.125
APTT	54.14±3.46	52.84±4.28	0.363

3.2.3 BMI and the mean of PT,INRandAPTT in GDM

The results obtained as shown in (Table 3.4) further indicates that hypercoagulability in GDM patients was dependent on body mass index. The Mean of PT and APTT of GDM patients with BMI >25 was insignificantly increase (19.72±4.35 and 53.59±4.03) respectively than those who had a normal body mass index <25. While the Mean of the INR of GDM patients with BMI >25was insignificant decrease than those who had a normal body mass index< 25. Hence there was a significant correlation between body mass index,GDM and hypercoagulability.

Table (3.4) shows the level of PT, INR and APTT in relation to BMI

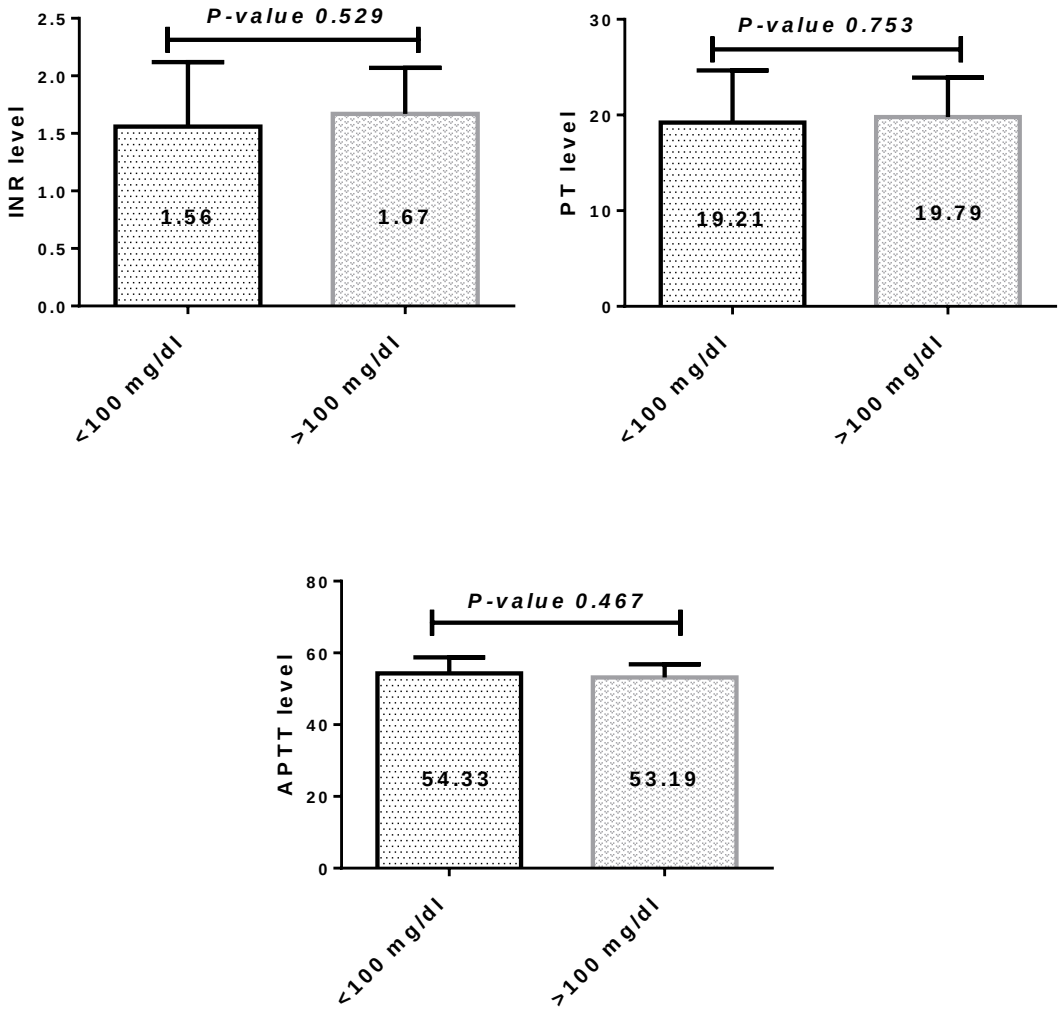
Parameters	<25 (Mean±SD)	>25 (Mean±SD)	*P-value*
PT	19.41±4.92	19.72±4.35	0.864
INR	1.67±0.49	1.62±0.44	0.771
APTT	53.42±3.69	53.59±4.03	0.909

3.2.4 FBG and the Mean of PT ,INR and APTT in GDM

An insignificant increase in PT of cord blood from GDM pregnant with FBG <100 (mg/dl) (19.21±5.45) to GDM pregnant women with FBG>100 (mg/dl) (19.79±4.11).Also the INR was insignificant increase in cord blood from GDM pregnant with FBG <100 (mg/dl) (1.67±0.40) to GDM pregnant women with FBG>100 (mg/dl) (1.56±0.56). Otherwise an insignificant increase in APTT of cord blood from GDM pregnant with

FBG <100 (mg/dl) (54.33±4.42) to GDM pregnant women with FBG>100 (mg/dl) (53.19±3.65), as shown in (Figure 3.1).

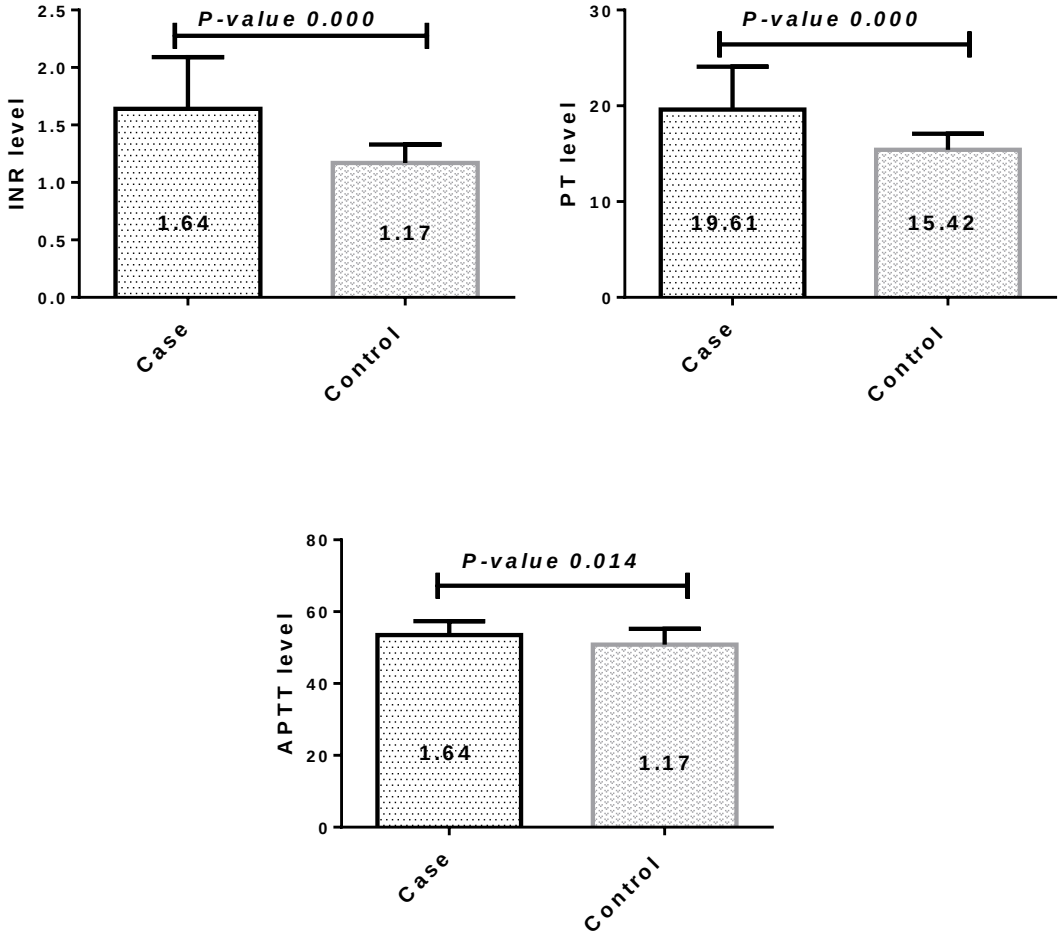
Figure (3.1): Mean Comparison of FBG in PT,INR and APTT



3.2.5 The Mean of PT, INR and APTT in GDM

Figure (3.2) illustrated the distribution of hemostatic profile according to. The mean of PT was significantly increased in pregnancy (19.61±4.47) when compared to the control group (15.42±1.67) *P. value=0.000*.Also the mean of INR was significant increase in pregnancy (1.64±0.45) compared to the control (1.17±0.16). In addition; the mean of APTT is also significantly increased inpregnancy(53.53±3.86) compared to control group (50.83±4.40) *P. value= 0.014*.

Figure (3.2) Mean Comparison of Study Parameters in Case versus the Control Group among GDM Women



3.2.6 Correlation between Gestational weeks and PT, INR and APTT

There was No correlation between gestational weeks and PT, INR and APTT (R-value =0.280)(R-value -0.236) (R-value 0.111) respectively as shown in table (3.5).

Table (3.5) Correlation between Gestational weeks and PT,INR and APTT

Variable		PT	INR	APTT
Gestational weeks	R-value	-0.280	-0.236	0.111
	P-value	0.135	0.209	0.558

3.2.7 Correlation between weight of baby and PT, INR and APTT

There was No correlation between weight of baby and PT, INR and APTT (R-value 0.069) (*R-value= 0.002) (R-value= -0.026)* respectively as shown in table (3.6).

Table (3.6) Correlation between weight of baby and PT, INR and APTT

Variable		PT	INR	APTT
Weight of baby	R-value	0.069	0.002	-0.026
	P-value	0.716	0.991	0.892

Chapter Four

4. Discussion, Conclusions and Recommendations

4.1 Discussion

Gestational DM is a systemic disease that affects both the mother and fetus. These patients are more likely to develop Type 2 DM; hence, they must be monitored closely. There are few studies in the available literature on PT and APTT in patients with GDM. GDM remains a significant cause of an increment in perinatal mortality, the risk of developing type II diabetes mellitus in the following 10 years increase by 20-30% .This study, aimed to determine PT, INR and APTT in cord blood of gestational diabetes mellitus mother, the two groups were comparable as regard to PT, INR and APTT. In the current study, 73% of gestational diabetic pregnant women aged<35 years whereas 26.7% of them were >35 Years. Also 53.3% of them had history of diabetes mellitus whereas 46.7% were not. On other hand 37% of them had family history of DM whereas 63% were not. On the other hand, 83% healthy pregnant women <35 years whereas 17% of them >35 Years Moreover, the results showed significant increase in BMI, FBG level and newborn weight among GDM mother *(P.value <0.05)* The Mean of GDM Patients who were hypercoagulable was significant increase in the age > 35 years (22.26±3.99) more than Age < 35 years (18.65±4.31) according to PT. It's also the same in INR result which was significant increase in the age

> 35 years (1.86±0.41) more than Age < 35 years (1.55±0.44). Otherwise the mean of APTTin newborns cord blood of GDM was significant increase in the age < 35 years (53.72±3.89) to the age > 35 years (53.01±3.94). Its show that there was a significant correlation between age and hypercoagulation. A significant increase in the Mean of GDM Patients that have history of diabetes (21.02±4.53) to the GDM Patients that haven't history of diabetes (18.00±3.94) , While there's No significant in the Mean of INR of GDM Patients that have history of diabetes (1.75±0.45) to the Patients that havn't history of diabetes (1.50±0.42). Also there's No significant in the Mean of APTT of GDM Patients that have history of diabetes (54.14±3.46) to the Patients that haven't history of diabetes (52.84±4.28). The Mean of PT and APTT of GDM patients with BMI >25 was insignificantly increase (19.72±4.35 and 53.59±4.03) respectively than those who had a normal body mass index <25. While the Mean of the INR of GDM patients with BMI >25was insignificant decrease than those who had a normal body mass index< 25. Hence there was a significant correlation between body mass index,GDM and hypercoagulability.

there was a significant gradual increased in PT in cord blood of GDM pregnant women (19.61±4.47) when compared to the control group (15.42±1.67) *P. value= 0.000*. Also there was significant increased in INR in cord blood of GDM pregnant women (1.64±0.45) when compared

to the control (1.17±0.16) *P. value= 0.000*. In addition; the APTT was also significantly increased in cord blood of GDM pregnant women (53.53±3.86) when compared to control group (50.83±4.40) *P. value= 0.014*. The results disagreed with the study Conducted by Zhao *et.al*, 2011 who reported that Prothrombin time (PT) of diabetic subjects was insignificantly shorter than that of non diabetic controls. In other hand the results of this study was agreed with the study conducted by Sarkar, S, *et al.,* (2005) who stated that no infant had a clinically apparent thrombotic event and there was no difference in IDM vs Controls. Also it was agreed with the study conducted by Gorar, S, *et al.*,(2016) who found in significantly lower PT and APTT in GDM patients compared to the controls (*P. value< 0.05*).On the other hand, there was an insignificant gradual decrease in PT (19.21±5.45) of cord blood from GDM pregnant women who their FBG <100 (mg/dl, while the PT of cord blood from GDM pregnant women who have FBG >100 (mg/dl) is increased (19.79±4.11). Also the same for INR which there is an insignificant gradual decrease in of cord blood from GDM pregnant women who their FBG <100 (mg/dl) (1.56±0.56), while the INR of cord blood from GDM pregnant women who have FBG >100 (mg/dl) is increased (1.67±0.40). But the APTT was an insignificant gradual increase cord blood from GDM pregnant women who their FBG<100(mg/dl)(54.33±4.42), while

the APTT of cord blood from GDM pregnant women who have FBG >100 (mg/dl) was decreased (53.19±3.65).

In this study there was no correlation between newborns cord blood PT and APTT in GDM mothers and gestational weeks, also there was no correlation between newborns cord blood PT and APTT in GDM mothers and weight of newborns.

In cord blood of Gestational diabetes mellitus (GDM), increasing of PT and APTT was recognized, and this will help to suggest that this group of Infants of Diabetic Mothers may be considered for special management and follow up for inflammation and hemostatic disorders. Hence, these parameters are potential useful markers for the early diagnosis of thromboembolic disorders.

This study findings could play an important role in the health impact of newborn of Diabetic Mothers. Furthermore, this study could be used as a reference or a benchmark study for related studies.

4.2 Conclusions

- Significant increased in PT, INR and APTT in cord blood gestational diabetes mellitus.
- Hemostatic parameter including PT, INR and APTT levels may be one of the valuable markers of hemostatic disorders in cord blood GDM.

- A significant reversed association between FBG level of GDM and in PT, INR and APTT.
- Estimation of in PT, INR and APTT may be considered as an easy, reliable, economic and rapid method.
- Newborn of GDM mother were at risk for thrombotic disorders.
- Cord blood is not a waste, and it has many benefits beside the clinical application.

4.3 Recommendations

1. The study provides an experimental evidence indicated that regular estimation of coagulation profile and platelet count for diabetes mellitus.

2.The role of changes in these parameters in the hemostatic system during diabetic pregnancy and the possible clinical relevance concerning the risk for thrombosis call for further studies.

3. More researches should be performed for monitoring the complete coagulation profile.

4. Further study with large sample size should be done to compare these parameters in diabetic patient and healthy individuals.

Reference

1. **Abdel HameedMirghani ,JohnDoupis.** Gestational Diabetes from A to Z. World J Diabetes.2017; 8(12):489-511.
2. **Adam SS, Key NS, Greenberg CS.** "D-dimer antigen: current concepts and future prospects". Blood. 2009;113 (13): 2878–2887.
3. **Amanda H., SerdarH, John T Repke**. Gestational Diabetes Mellitus. Rev Obstet Gynecol. 2008; 1(3): 129–134.
4. **American College of Obstetricians and Gynecologists (ACOG).** Committee on Practice Bulletins-Obstetrics. Practice Bulletin No. 137: Gestational diabetes mellitus. Obstet Gynecol.2013;122:406-16.
5. **Avagliano L, Marconi AM, Candiani M, Barbera A, Bulfamante G.** Thrombosis of the umbilical vessels revisited. An observational study of 317 consecutive autopsies at a single institution. Hum Pathol 2010; 41: 971–979.
6. **Blanco, M.V., Vega, H.R., Guerri-Guttenberg, R.A., Giuliano, Grana, D.R., Azzato, F. and Milei, J.**, 2011. Histopathology and histomorphometry of umbilical cord blood vessels. Findings in normal and high risk pregnancies,*5*(2), pp.50-57.
7. **Chamberlain C, McNamara B, Williams ED, Yore D, Oldenburg B, Oats J, Eades S.** Diabetes in pregnancy among indigenous women

in Australia, Canada, New Zealand and the United States. Diabetes Metab Res Rev.2013; 29:241–256.

8. **Crowther CA, Hiller JE, Moss JR, *et al.*** for Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group, authors. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med. 2005;352:2477–2486.
9. **Edstrom, C. S and Christensen, R. D.** Evaluation and treatment of thrombosis in the neonatal intensive care unit. *ClinPerinatol*, 2000. 27**,** 623-41.
10. **Eman M., Alfadhali**. Gestational diabetes mellitus. Saudi Medical Journal.2015; 36(4);399-406.
11. **Gabbe SG, Graves CR.** Management of diabetes mellitus complicating pregnancy. Obstet Gynecol. 2003;102:857–868.
12. **Gale AJ.** Current understading of Hemostasis. *ToxicolPathol*2011; 39(1): 273–80.
13. **Gorar, S., Alioglu, B., Ademoglu, E., Uyar, S., Bekdemir, H., Candan, Z., Saglam, B., Koc, G., Culha, C. and Aral, Y., 2016**. Is there a tendency for thrombosis in gestational diabetes mellitus?. *Journal of laboratory physicians*, *8*(2), p.101.
14. **Gruel Y**. Specificities of neonatal hemostasis and implications in pathologic situations.Arch Pediatr, 2010

15. **Habibollah, S.**, 2010. Cord and cord blood stem cell tissue engineering for therapeutic intervention in liver disease.

16. **Hall JE** (2010). Guyton and Hall Textbook of Medical Physiology: Enhanced E-Book. 11th ed. Philadelphia: Elsevier Health Sciences; Hemostasis and blood coagulation; pp. 457–9.

17. **Hedderson M, Ehrlich S, Sridhar S, Darbinian J, Moore S, Ferrara A.** Racial/ethnic disparities in the prevalence of gestational diabetes mellitus by BMI. Diabetes Care.2012;35:1492–1498.

18. **Hoffbrand, Victor, and Paul Moss**. Essential haematology. 6th edition. John Wiley & Sons;2011.

19. **Kumar V, Abbas AK, Fausto N, Aster JC.** Hemodynamic disorders, thromboembolic disease and shock. In: Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia, PA: Saunders Elsevier; 2010. p. 118-20.

20. **Lemkes BA, Hermanides J, Devries JH, Holleman F, Meijers JC, Hoekstra JB(2010).** Hyperglycemia: A prothrombotic factor. *J ThrombHaemost*;8:1663‑9.

21. **Leung LLK**. Overview of hemostasis: UptoDate. 2016.06. *Patient education materials* [Internet] [cited 2016 Feb 12] Available from: https://www.uptodate.com/contents/overview-ofhemostasis

22. **Lippi G, Salvagno GL, Rugolotto S, *et al.*** Routine coagulation tests in newborn and young infants. J Thromb Thrombolysis 2007;24:153-5.

23. 3. **Marion DW**. Screening and diagnosis of gestational diabetes mellitus. [Accessed August 29, 2008]. UpToDate Web site. http://www.uptodate.com.

24. **Metzger BE, Lowe LP, Dyer AR, *et al.*** for HAPO Study Cooperative Research Group, authors. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358:1991–2002. [PubMed]

25. **Neary E, McCallion N, Kevane B, Cotter M, Egan K, Irene R, Áinle FN.** To Characterize Standard Laboratory Coagulation Parameters and Plasma Thrombin Generation in Very Preterm Infants and to Investigate Their Relationship to Clinical Outcomes.

26. **Owens AP(2010),** 3rd, Mackman N. Tissue factor and thrombosis: The clot starts here. ThrombHaemost. 104:432–9.

27. **Palta S, Saroa R, Palta A.** Overview of the coagulation system. *Indian J Anaesth*2014; 58(5):515–23.

28. **Pichler E, *et al***. The neonatal coagulation system and the vitamin K deficiency bleeding. Wien Med Wochenschr. 2008.

29. **Sarkar, S., Hagstrom, N.J., Ingardia, C.J., Lerer, T. and Herson, V.C., 2005**. Prothrombotic risk factors in infants of diabetic mothers. *Journal of perinatology*, *25*(2), p.134.

30. **Tanaka, K.A., Key, N.S. and Levy, J.H., 2009**. Blood coagulation: hemostasis and thrombin regulation. *Anesthesia & Analgesia*, *108*(5), pp.1433-1446.

31. **Thornton P, Douglas J.** Coagulation in pregnancy (2010). Best Pract Res ClinObstetGynaecol.; 24:339–52.

32. **Thomas A. Bu chanan and Anny H. Xiang .** Gestational diabetes mellitus. *J clinical investigation*2005;115(3):485-491.

33. **Ulla K., Lene R., Gitte O., Ditte S., Niels M., Per O.** Gestational diabetes: A clinical update. World J Diabetes. 2015 Jul 25; 6(8): 1065–1072.

34. **US Preventive Services Task Force, authors.** Screening for gestational diabetes mellitus: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2008;148:759–765.

35. **Young-Ho Lee.** Implication of Cord Blood for Cell-Based Therapy in Refractory Childhood Diseases. Int J Stem Cells. 2010 May; 3(1): 22–28.

36. **Znamens'ka TK, Zhdanovych OI, Shevchenko LI, Kurylina TV, Medvedenko HF, Bieliavs'ka TO, Matviienko NO, Kolomiĭchenko TV.** The coagulation link of the hemostatic system in newborn infants from mothers with extragenital pathology. LikSprava. 2000 Jul-Aug;(5):54-5.

Appendices

Sudan University of sciences and technology

Collage of higher education

Collage of medical laboratory sciences

Hematology department

Assessment of Prothrombin Time and Activated Prothrombin Time in Umbilical Cord Blood of Gestational Diabetic Mothers

Appendix I:Questionnaire

Name: ……. ID code……..

Gender of baby:……...

Age:…….

BMI…….

Medication of the mother? .....

Gestational Diabetes onset?.....

Weeks of gestation?.....

History of thrombosis?.....

History of abortion?.....

Gestational weeks of abortion?.....

Weeks of gestation on delivery time? .....

Weight of newborn? .....

Apgar score: ……

Health problem of newborn?.....

Maternal Glucose Level: …….

After understanding the contents of this questionnaire and the aim of research I agree …… ……. To collect the sample.

The researcher admitted not to use theblood sample in any other porpoises.

Signature:…….**Date**…….....

<u>Laboratory investigation:</u>

PT:…….INR:……..

APTT:……..

Appendix II Biomed Leaflet for APTT

BioMed-Liquicellin-E for APTT determination

BIOMED DIAGNOSTICS

Activated Partial Thromboplastin Time

REF: PTT202180 (6x3 ml)

INTENDED FOR USE:

For quantitative determination of Partial the **Biomed- LIQUICELIN-E** activity in plasma

PRINCIPLE:

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions.
APTT is prolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

SPECIMEN COLLECTION:

The arrest of bleeding depends upon primary platelet plug formed along with the formation of stable fibrin clot. Formation of this clot involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

Activated Partial **Biomed- LIQUICELIN-E** Time is prolonged by a deficiency of coagulation factors of the intrinsic pathway of the human coagulation mechanism such as factor XII, XI, VIII, X, V, II and Fibrinogen.
Determination of APTT helps in estimating abnormality in most of the clotting factors of the intrinsic pathway including congenital deficiency of factor VIII, IX, XI and XII and is also a sensitive procedure for generating heparin response curves for monitoring heparin therapy.

REAGENT COMPOSITIONS :

Biomed-LIQUICELIN-E is liquid ready to use activated cephaloplastin reagent for the determination of Activated Partial Thromboplastin Time. It is a phospholipids preparation derived from rabbit brain with ellagic acid as an activator.
Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

PACKAGE: Collection and storage.

- Store the reagent at 2-8°C. DO NOT FREEZE.
- The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The reagent is stable at 2-8°C up to the expiration date.

PRECAUTIONS & WARNING :

Avoid pipetting with mouth.
The preparation, according to current regulation, is classified as not dangerous.
The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.
Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

REAGENT PREPARATION & STABILITY :

- Use freshly collected blood taken into 0.11 mol/L trisodium citrate in the ratio 9 parts blood to 1 part anticoagulant.
- Centrifuge immediately for 5 minutes at RCF 1500-2000 g and separate plasma into a clean test tube.
- Plasma should be tested within 3 hours (keep refrigerated).

REQUIRED MATERIALS NOT PROVIDED:

(a) 12 X 75 mm glass test tubes. (b) 0.1 ml. (c) Stop watch. (d) Water bath or heating block at 37°C. (e) Fresh Normal Pooled Plasma. (f) CaCl2 (0.02 mol/l).

PROCEDURE:

Manual method

1. Pre-incubate the Calcium Chloride Reagent to 37°C for at least 10 minutes. Pipette 100µl of test or control plasma into a test cuvette.
2. Incubate the plasma at 37°C for 1 to 2 minutes.
3. Pipette 100µl of the APTT reagent, into reagent cuvette containing the plasma. Maintain the suspension of the APTT reagent by magnetic stirring or mixing by inversion immediately prior to use.
4. Incubate at 37°C for 5 minutes.
5. Add 100µl preincubated Calcium Chloride solution and simultaneously start the timer.
6. Record the clotting time in seconds.

Calibration Curve Method (For determination of heparin concentration):

1. Dilute heparin (as used for treatment) with physiological saline to a concentration of 10 U/ml.
2. Mix 0.2 ml of 10 U/ml diluted heparin with 1.8 ml of FNP to give a heparin standard of 1U/ml concentration.
3. Dilute the heparin standard as prepared above (1U/ml) with FNP as follow

Test tube no.	1	2	3	4	5	6	7
Heparin standard (1U/ml) in ml	0.5	0.4	0.3	0.2	0.1	0.1	
FNP in ml		0.1	0.2	0.3	0.4	0.9	0.5
Heparin concentration (U/ml)	1.0	0.8	0.6	0.4	0.2	0.1	0.0

4. Pipette 0.1 ml each of the seven heparin dilutions into clean test tubes.
5. Add 0.1 ml LIQUICELIN-E reagent to each test tube.
6. Mix well and incubate each test tube at 37°C for exactly 3 minutes before testing.
7. Forcibly add 0.1 ml calcium chloride (prewarmed at 37°C) to each test tube, one by one and simultaneously start the stopwatch.
8. Gently tilt the tube back and forth and stop the stopwatch as the first fibrin strand is visible and the gel/clot formation begins. Record the time in seconds.
9. Repeat steps 4-8 for each dilution for duplicate test, and find the average of the duplicate test values.
10. Plot the mean of the double determination in seconds, against each heparin concentration using BioMed-**LIQUICELIN-E** graph paper.
11. Clotting times (APTT) of test specimens can be interpolated against the heparin concentration to determine the heparin concentration of the sample in U/ml.

CALCULATION:

Manual Method

(a) The results may be reported directly in terms of the mean of the double determination of APTT of the test plasma.

(b) Or as a ratio 'R': $R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{APTT of FNP (in seconds)}}$

Calibration Curve Method

Heparin concentration in the test sample can be directly obtained from the Biomed- **LIQUICELIN-E** calibration curve by interpolating the test plasma clotting time against the heparin concentration in U/ml.

EXPECTED VALUE:

Normal values using **LIQUICELIN-E** reagent are between 28-40 seconds at 3 minutes activation time. Between manual and Turbo densitometric instrument results a variation of 1-2 seconds may be expected. For photo optical instruments, it is recommended that each laboratory must establish its own normal range.

WASTE DISPOSAL:

The disposal of the product must be in accordance with local regulation concerning waste disposal.

PERFORMANCE:

1. Due to inter and intra laboratory variations users must establish their own normal population range as well as normal and abnormal range.
2. It is recommended that controls with known factor activity should be run simultaneously with each test series routinely.
3. Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
4. Incorrect dilution of heparin is also a potential source of error.
5. Oxalated plasma may include prolonged clotting times.
6. Clotting time of patients on anticoagulant therapy depends upon the time lag between the specimen collected and the last dose.
7. Abnormalities of coagulation factor VII, factor XIII and platelets are not detected by this test procedure.
8. For automated equipment it is strongly recommended that the equipment manufacturer methodology be strictly adhered to.
9. In heparin monitoring time of collection of blood sample is important since the in-vitro half-life of heparin is approximately 1.5hours. When it is administered intravenously it has an immediate anti coagulant effect but its efficacy decreases rapidly with time.
10. Platelet factor IV, a heparin-neutralizing factor can be released due to platelet aggregation or damage. In order to prevent this phenomenon in-vitro the specimen should be collected with a minimum of trauma.
11. Decrease in APTT time is observed in males under estrogen therapy and oral contraceptive administration in females.

LIMITATIONS :

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. **LIQUICELIN-E** reagent is not from human source hence contamination due to HBsAg and HIV is practically excluded.
3. Reagent contains 0.01% Thimerosal as preservative.
4. It is very important that clean and dry micropipette tips be used to dispense the reagent.
5. Avoid exposure of the reagent to elevated temperatures, contamination. Immediately replace cap after use and store at recommended temperatures only.

REFERENCES:

1. Biggs R. and R.G. McFarlane: Human Blood Coagulation and its Disorders, Blackwell Scientific Publications, Oxford,1962.
2. Hoffmann, J.J.M.L. and Neulendijk P.N.: Thrombos. Haemosta. (Stuttgart) 39, 640 (1978).
3. CRC, Handbook Series in Clinical Laboratory, Science, Section 1: Haematology. Volume III, 1980. CRC Press, Inc. Boca Raton, Florida

	Consult Instructions for Use
	Caution, Consult accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
	Use by

Egy Chem for lab technology

EGY- CHEM for lab technology
Badr City, Industrial Area Piece 170
250 Fadan In East of Elrubaki, EGYPT
Office Tel: +202 26236727 / +202 26236598
Factory Tel: +202 23108170 / +202 23108171
Fax: +202 26240986
www.egy-chem.com

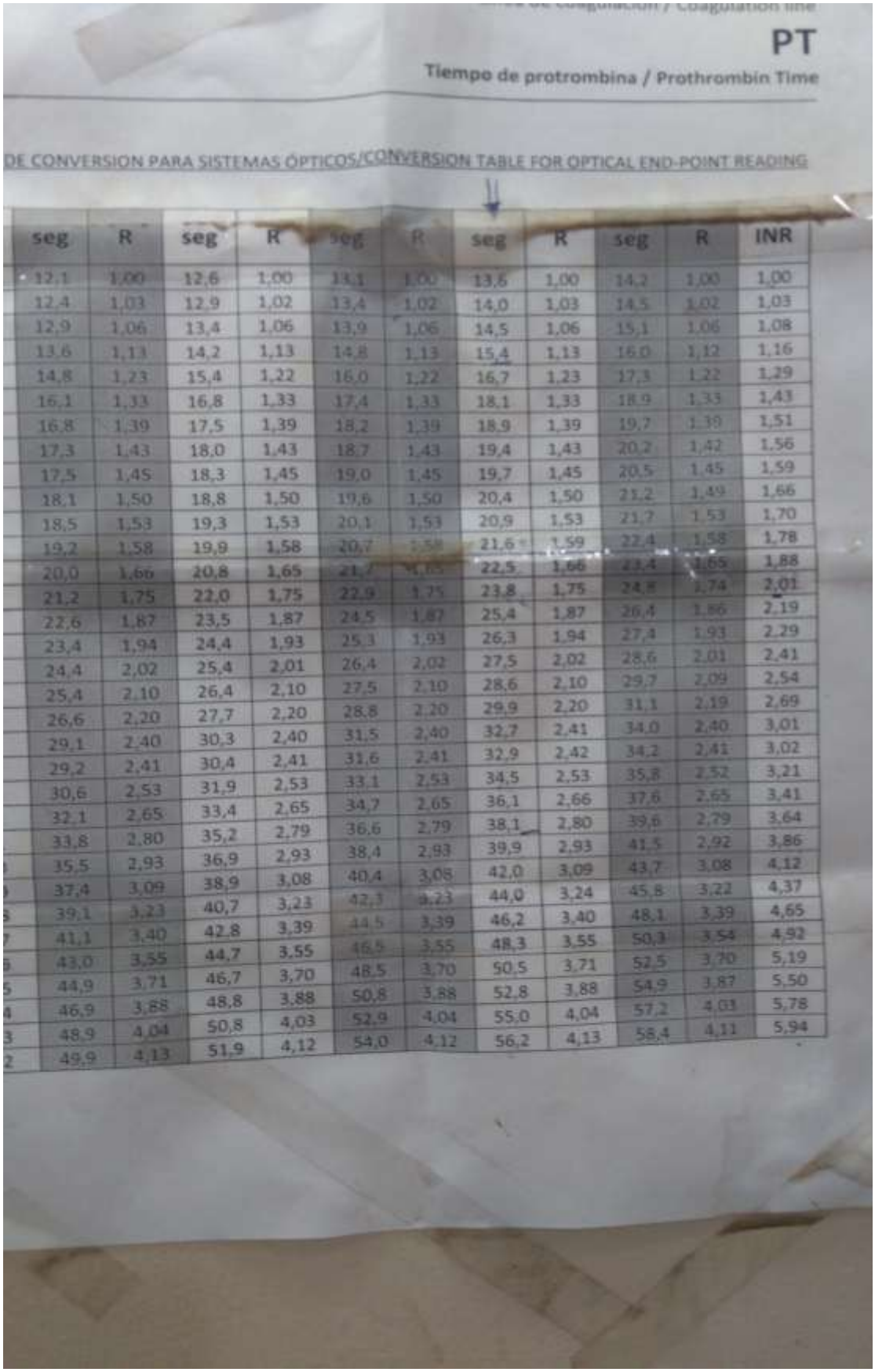
CE
EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

Appendix III: Images of Device and Reagents

CL -2000 Biobase automated Coagulometer

PT and APTT Biomed Reagent

Appendix IV: INR Sheet



PT

Tiempo de protrombina / Prothrombin Time

DE CONVERSION PARA SISTEMAS ÓPTICOS/CONVERSION TABLE FOR OPTICAL END-POINT READING

seg	R	seg	R	seg	R	seg	R	seg	R	INR
12,1	1,00	12,6	1,00	13,1	1,00	13,6	1,00	14,2	1,00	1,00
12,4	1,03	12,9	1,02	13,4	1,02	14,0	1,03	14,5	1,02	1,03
12,9	1,06	13,4	1,06	13,9	1,06	14,5	1,06	15,1	1,06	1,08
13,6	1,13	14,2	1,13	14,8	1,13	15,4	1,13	16,0	1,12	1,16
14,8	1,23	15,4	1,22	16,0	1,22	16,7	1,23	17,3	1,22	1,29
16,1	1,33	16,8	1,33	17,4	1,33	18,1	1,33	18,9	1,33	1,43
16,8	1,39	17,5	1,39	18,2	1,39	18,9	1,39	19,7	1,39	1,51
17,3	1,43	18,0	1,43	18,7	1,43	19,4	1,43	20,2	1,42	1,56
17,5	1,45	18,3	1,45	19,0	1,45	19,7	1,45	20,5	1,45	1,59
18,1	1,50	18,8	1,50	19,6	1,50	20,4	1,50	21,2	1,49	1,66
18,5	1,53	19,3	1,53	20,1	1,53	20,9	1,53	21,7	1,53	1,70
19,2	1,58	19,9	1,58	20,7	1,58	21,6	1,59	22,4	1,58	1,78
20,0	1,66	20,8	1,65	21,7	1,65	22,5	1,66	23,4	1,65	1,88
21,2	1,75	22,0	1,75	22,9	1,75	23,8	1,75	24,8	1,74	2,01
22,6	1,87	23,5	1,87	24,5	1,87	25,4	1,87	26,4	1,86	2,19
23,4	1,94	24,4	1,93	25,3	1,93	26,3	1,94	27,4	1,93	2,29
24,4	2,02	25,4	2,01	26,4	2,02	27,5	2,02	28,6	2,01	2,41
25,4	2,10	26,4	2,10	27,5	2,10	28,6	2,10	29,7	2,09	2,54
26,6	2,20	27,7	2,20	28,8	2,20	29,9	2,20	31,1	2,19	2,69
29,1	2,40	30,3	2,40	31,5	2,40	32,7	2,41	34,0	2,40	3,01
29,2	2,41	30,4	2,41	31,6	2,41	32,9	2,42	34,2	2,41	3,02
30,6	2,53	31,9	2,53	33,1	2,53	34,5	2,53	35,8	2,52	3,21
32,1	2,65	33,4	2,65	34,7	2,65	36,1	2,66	37,6	2,65	3,41
33,8	2,80	35,2	2,79	36,6	2,79	38,1	2,80	39,6	2,79	3,64
35,5	2,93	36,9	2,93	38,4	2,93	39,9	2,93	41,5	2,92	3,86
37,4	3,09	38,9	3,08	40,4	3,08	42,0	3,09	43,7	3,08	4,12
39,1	3,23	40,7	3,23	42,3	3,23	44,0	3,24	45,8	3,22	4,37
41,1	3,40	42,8	3,39	44,5	3,39	46,2	3,40	48,1	3,39	4,65
43,0	3,55	44,7	3,55	46,5	3,55	48,3	3,55	50,3	3,54	4,92
44,9	3,71	46,7	3,70	48,5	3,70	50,5	3,71	52,5	3,70	5,19
46,9	3,88	48,8	3,88	50,8	3,88	52,8	3,88	54,9	3,87	5,50
48,9	4,04	50,8	4,03	52,9	4,04	55,0	4,04	57,2	4,03	5,78
49,9	4,13	51,9	4,12	54,0	4,12	56,2	4,13	58,4	4,11	5,94